



Impact of different dietary protein sources on performance, litter quality and foot pad dermatitis in broilers

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ABSTRACT. The aim of this study was to evaluate potential influences of different dietary protein sources on performance, litter quality and health of foot pads in broilers. In total, 160 one-day-old chickens were divided into four dietary groups at day 8. Four different diets were prepared as follows: the level of soyabean meal in the control diet amounted 32.5% (SBM), while in other diets soyabean meal was partly replaced by rapeseed meal 14.5% (RSM); haemoglobin meal 4.5% (HBM) or algae meal 4.0% (ALG). Body weight was measured weekly. Measurement of dry matter content of litter and excreta as well as external assessment of foot pads were performed weekly. Birds fed the RSM diet had the highest body weight at day 44 in comparison to animals fed other experimental diets. Broilers fed the HBM diet had the lowest body weight at the end of the trials. Foot pad dermatitis (FPD) severity was significantly higher in birds fed RSM and ALG diets in comparison with those fed SBM or HBM diets at day 44 of life. In conclusion, using RSM could be the most suitable alternative protein source due to higher body weight gains. However, further studies are required to optimize the diet composition which could simultaneously improve foot pad health.

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Introduction

World demand for protein is rising in line with the growing human population. According to Food and Agriculture Organization of the United Nations (FAO, 2013) the global food demand of 2013 will double in 2050, particularly for animal protein (meat and dairy products). In poultry nutrition, due to high protein accretion, there is a need to supplement this substance because it plays essential roles in the body and is needed for growth. The amino acids requirements for broilers are high, especially during the early stages of life (Beski et al., 2015). Soyabean meal (SBM) is the dominant protein source used in poultry diets due to its favourable nutrient composition

and its well-balanced amino acids profile. It is also an excellent source of energy not only for poultry but also for swine. Thus, the high protein content, with its well-balanced and highly digestible amino acids, makes SBM a valuable protein for human nutrition and animal feeding (Kocher et al., 2002).

There are market trends towards the use of cheaper raw materials, protein sources of higher sustainability, and products increasing animal health characteristics (Laudadio and Tufarelli, 2011; van der Spiegel et al., 2013). Soyabean meal is the gold standard to which alternative protein sources are compared. Some important aspects for using, alternative protein sources for livestock nutrition should be considered. Among them are performance and health of animals

as well as food safety that should not be adversely affected.

Rapeseed meal (RSM) has been used as a feed ingredient worldwide for many years in such animal species as poultry, swine, cattle and fish (Nagel et al., 2012). According to FAO (2016) the world RSM production amounted 39.1 million tonnes in 2015/2016, and in 2014 the main producer of RSM was the European Union (13.9 million tonnes). The amino acid balance of RSM compares well with that of SBM (Clandinin, 1967), the former being higher in methionine and the latter in lysine. Blood meal, an important animal protein by-product, contains over 800 g protein · kg⁻¹ dry matter (DM), 90 g total lysine · kg⁻¹ DM and small amounts of ash and lipids (Donkoh et al., 2001).

In poultry diets, dried algae up to a level of 5–10% can be used as partial replacement for conventional proteins (Becker, 2004). The yellow colour of broiler skin, shanks and egg yolk is an interesting effect of feeding algae (important for some regional markets in the world) (Becker, 2004; Pulz and Gross, 2004). Chemical composition of algae is well documented. They mainly contain proteins (28–71% DM), carbohydrates (10–57% DM), lipids (4–22% DM), vitamins, trace elements and antioxidants (Becker, 2004; Yaakob et al., 2014). However, the nutritive value of microalgae is determined mainly by the protein content. Also, highly unsaturated fatty acids (e.g., eicosapentaenoic acid, arachidonic acid and docosahexaenoic acid) content is essential (Spolaore et al., 2006). It was estimated that *Chlorella* and *Spirulina* algae are the most popular in the market (Pulz and Gross, 2004).

Litter quality is considered to be a major factor in the development of foot pad dermatitis (FPD) along with nutrition (Kamphues et al., 2011; Taherparvar et al., 2016). Various dietary nutrients/ingredients can affect the litter quality and hence the foot pad health. The indigestible carbohydrate levels in plant sources (primarily SBM) are remarkably high, about 15–20% DM (Choct, 1997; Choct et al., 2010). These carbohydrates are known as non-starch-polysaccharides (NSP) and are also found at high concentrations in wheat and other grains affecting the digestive process and litter quality (Choct, 1997; Youssef et al., 2011). The higher dietary NSP concentrations the higher viscosity of digesta which results in 'sticky' excreta adhering to bird foot pads (Youssef et al., 2011). Moreover, SBM is known for its high potassium content (>20 g · kg⁻¹ DM), which leads to an increase in water intake and moisture of excreta (Youssef et al., 2011).

FPD poses a challenge for poultry production, potential effects on productivity, animal welfare and economics (Abd El-Wahab et al., 2011, 2012a). FPD is affected by several factors; among them are litter quality, diet composition and intestinal diseases (Abd El-Wahab et al., 2011, 2012a, 2012b, 2013a, 2013b; Youssef et al., 2011). The foot pad lesions may be painful, particularly in severe cases, and together with a worsening state of an animal constitute a serious problem. Also, through the lesions bacteria or other pathogens can get into animal organism and as a result cause partial or total condemnation of the carcass (Ekstrand et al., 1997). Nevertheless, feeding measures and housing conditions are as important as managing the quality of litter, especially its moisture content (the key factor) in reducing the incidence and severity of FPD.

The aim of the present study was to evaluate the potential effects of different dietary protein sources and levels on performance, litter quality and health of foot pads in broilers.

Material and methods

Animal experiments were carried out according to German regulations. A notification or an approval according to the Animal Protection Act were not needed to perform the experiments.

Animals and housing

In total, 160 one-day-old chickens (ROSS-708, as-hatched) were obtained from a commercial hatchery. Before starting the feeding trials at day 8, all birds were housed in four identical floor pens littered with wood shavings that were kept very dry and clean to prevent any foot pad lesions, especially during the first week of age. Birds were fed a commercial pelleted starter diet (Deuka, Deutsche Tiernahrung Cremer GmbH & Co. KG, Düsseldorf, Germany) *ad libitum* that covered birds requirements. A temperature of 34–36 °C at the first day of age was ensured by infrared lamps and was lowered by 1 °C every two days. The photoperiod from day 4 was 16 h of light and 8 h of darkness.

Experimental design

At the beginning of the second week, animals were randomly allocated to four groups with two replicate pens of 20 birds each. All animals were wing-tagged. All eight pens were identical in size (2 × 1 m), littered with wood shavings (1 kg · m⁻²; 88.3 ± 0.2% DM). Four different diets were prepared for this experiment. The level of soyabean meal (SBM) in the

Table 1. Chemical composition of starter and experimental diets fed to broilers, %

Indices, %	Starter (days 0–7) all groups	Treatment (days 8–44) ¹			
		SBM	RSM	HBM	ALG
Wheat	+	48.9	40.5	34.6	38.6
Maize	+	9.00	12.5	28.4	19.8
Soyabean meal	+	32.5	22.9	22.5	28.0
Rapeseed meal	–	–	14.5	–	–
Haemoglobin meal	–	–	–	4.50	–
Algae meal	–	–	–	–	4.00
Fat/oil	+	5.20	5.20	5.20	5.20
CaCO ₃	+	1.80	1.80	1.80	1.80
Dicalcium phosphate	–	1.00	1.00	1.37	1.00
Sodium chloride	+	0.35	0.35	0.35	0.35
Lysine	–	0.27	0.25	0.20	0.28
Methionine	+	0.16	0.15	0.19	0.15
Threonine	–	0.10	0.10	0.10	0.10
Trace elements supplement ²	+	0.30	0.30	0.30	0.30
Vitamin supplement ³	+	0.30	0.30	0.30	0.30

¹ treatment: SBM – diet with only soyabean meal, RSM – diet with rapeseed meal, HBM – diet with haemoglobin meal, ALG – diet with algae meal; ² added to the diet per kg: mg: Cu 22.5, Zn 150, I 1.50, Se 0.45; ³ added to the diet per kg: IU: vit. A 12,000, vit D₃ 5000; mg: vit. E 25; µg: biotin 200; due to statistical approximation the sum of diets might not be 100%

control diet amounted 32.5%, while in other diets SBM was partly replaced by rapeseed meal (RSM) – 14.5%; haemoglobin meal (HBM) – 4.5%; or algae meal (ALG) – 4% (Table 1). It has to be stressed that the phytase, NSP-enzymes and anticoccidial drugs were not used in all experimental diets. The diets were offered in circular troughs and water was in bell drinkers. Both were available *ad libitum*. Samples of the four diets were analysed by standardised laboratory methods (VDLUFA, 2012). The composition and the chemical analysis of the experimental diets are shown in Tables 1 and 2, respectively.

Performance parameters

Feed and water intakes were measured daily at pen level. Individual body weight (BW) of the animals was recorded weekly. The feed conversion ratio (FCR) was calculated on the basis of feed consumed throughout the experimental period.

Excreta and litter quality

The fresh excreta were collected from each pen once a week by putting a pond liner in each pen for one hour until about 70–100 g of fresh pure excreta per pen had been obtained. The collected excreta (sampling during ~1 h) were then removed from each pen, thoroughly mixed and dried at 103 °C to determine the DM content.

Litter samples for measuring DM content were collected weekly from five sites (the four corners and the middle of the pen), then thoroughly mixed. Subsamples of about 100 g were taken to assess moisture content by drying at 103 °C until a constant mass was obtained (VDLUFA, 2012).

FPD scoring criteria

The external scoring was performed for birds at the beginning of the experiment (day 8), then weekly till day 44. If the feet were dirty they were gently washed with a wet cloth and dried before scoring; only the central plantar area was scored. Signs of foot pad lesions were recorded on a 7-point scale (0 = normal skin; 7 = over half of the foot pad is covered with necrotic scales) according to Mayne et al. (2007).

Statistical analysis

The statistical analysis of the collected data was performed using the Statistical Analysis System for Windows, the SAS[®] Enterprise Guide[®] version 7.1 (SAS Institute Inc., Cary, NC, USA). The collected data (individual performance data as well as foot pad scores) were analysed by means of one-factorial analysis of variance for normally distributed data with the individual as the replicate. Results of $P < 0.05$ were treated as statistically significant. However, it has to be pointed out that feed and water intakes, FCR, as well as DM content in excreta and litter were estimated only on pen level, and therefore could not be statistically analysed due to the low number.

Results

Chemical analyses of diets

Chemical analyses of the different experimental diets are shown in Table 2. The results indicated similar energy content (13.4–14.1 MJ · kg⁻¹ DM) and protein content (228–235 g · kg⁻¹ DM) for all experimental diets (days 8–44). However, there was a decrease in the potassium level in the HBM diet (7.66 g · kg⁻¹ DM vs 9.83 g · kg⁻¹ DM for the SBM diet). No marked differences in the sodium content were observed between the experimental diets (1.95–2.13 g · kg⁻¹ DM).

Feed intake and performance

At the beginning of the experiment (day 8), no significant differences in BW were noted between the experimental groups (birds were randomly distributed to the pens). It should be stressed that

Table 2. Chemical analyses of all diets fed to broilers

Indices	Starter (days 0–7)	Treatment (days 8–44) ¹			
	all groups	SBM	RSM	HBM	ALG
Chemical analyses, g · kg ⁻¹ DM					
crude ash	59.6	64.8	69.2	63.3	65.5
crude fat	59.1	80.1	84.5	83.3	89.6
crude fibre	21.8	40.2	46.7	30.4	35.1
crude protein	252	231	228	235	235
starch	404	386	368	422	402
sugar	59.1	51.8	62.4	39.2	45.6
Ca	10.8	14.0	14.7	15.7	14.4
P	7.84	5.05	7.16	6.79	6.77
K	11.0	9.83	8.93	7.66	9.31
Na	1.53	1.95	2.13	1.97	1.96
Cl	1.98	4.18	4.09	3.99	4.14
arginine	15.1	15.0	14.4	13.0	14.7
cysteine	5.44	4.31	4.28	3.87	4.16
lysine	16.4	14.1	13.8	15.4	14.1
methionine	4.32	5.23	5.19	4.97	5.74
phenylalanine	12.6	10.6	10.9	12.3	11.1
threonine	10.4	8.63	8.93	8.01	8.84
valine	10.2	9.97	10.5	12.4	10.8
glycine	10.6	9.53	10.1	9.80	9.81
serine	13.1	11.3	11.3	10.9	11.3
tyrosine	8.75	7.62	7.52	7.10	7.69
Metabolizable energy, MJ · kg ⁻¹ DM	13.2	13.5	13.4	14.1	14.0

¹see Table 1

the total feed intake was corrected for mortality (Table 3). Birds fed the RSM diet had a markedly higher feed intake (4 656 g) than other experimental groups. However, birds fed the HBM diet had the numerically lowest feed intake (3 859 g). Regarding the total water intake, birds fed the RSM diet had a numerically higher total water intake (11 616 g)

Table 3. Feed and water intakes and growth performance of broilers fed diets with different protein sources and combinations

Indices	Treatment ¹			
	SBM	RSM	HBM	ALG
Total feed intake (days 8–44), g · bird ⁻¹	4368	4656	3859	4196
Total water intake, 11048 (days 8–44), g · bird ⁻¹	11048	11616	9796	11196
Water : feed intake (days 8–44)	2.53	2.49	2.53	2.68
Body weight (day 7), g	180.7 ± 14	184.3 ± 11.3	182.4 ± 12.1	180.4 ± 15.7
Body weight (day 44), g	2769 ± 319 ^b	2909 ± 325 ^a	2478 ± 320 ^c	2739 ± 378 ^b
FCR (days 8–44)	1.68	1.71	1.68	1.64

¹see Table 1; ^{abc} – means within the same row with different superscripts are significantly different at $P < 0.05$

than other groups, while birds fed HBM had a very low total water intake (9 796 g). The water:feed intake ratio was numerically higher for groups fed the ALG diet (2.68), while the numerically lowest ratio was recorded in groups fed the RSM diet (2.49). The water:feed intake ratio was similar between groups fed SBM and HBM diets (2.53).

Significant differences were observed in the final BW between the experimental groups (Table 3). Birds fed the RSM diet had a significantly higher BW (2 909 g) in comparison to other experimental groups. No significant differences in the final BW (day 44) were noted between groups fed ALG and SBM diets (2 739 and 2 769 g, respectively). Birds fed the HBM diet had a significantly the lowest BW (2 478 g).

Furthermore, the FCR was almost equal between groups fed SBM and RSM diets (68 and 71, respectively), while the numerically most favourable FCR was recorded for groups fed the ALG diet (64).

Excreta and litter quality

The numerically highest mean DM content for excreta was found in birds fed the RSM diet (16.4%), while the lowest mean DM content for excreta was noted in those fed the ALG diet (14.7%) (Table 4). No marked differences were found between groups fed the HBM and SBM diets (15.3 and 15.6%, respectively).

Regarding litter DM contents, birds fed the HBM diet had the numerically highest mean DM content (51.1%) (Table 4). The lowest mean DM content was noted in birds fed the SBM diet (41.8%). However, no marked differences were observed between birds fed the RSM and ALG diets (45.6 and 46.8%, respectively). Again, birds fed the SBM diet had numerically the lowest final litter DM content (44.8%).

FPD lesions

At the beginning of the experiment (day 8) there was no evidence of external FPD lesions. Birds fed either RSM or ALG diets had significantly ($P < 0.05$) the highest FPD scores (6.28 and 6.58, respectively) at day 44 (6 week) of life (Table 5). No significant differences in FPD scores were found between birds fed either SBM or HBM diets (5.42 and 5.48, respectively) at day 44 of life. To give an idea regarding clinical relevance, the prevalence of high/severe FPD scores (6–7) amounted to 76 and 88% for birds fed RSM and ALG diets vs 46 and 56% for those fed SBM and HBM diets, respectively.

Table 4. Dry matter (DM) contents of excreta and litter in broilers fed diets with different protein sources and combinations, %

Indices	Treatment ¹			
	SBM	RSM	HBM	ALG
DM contents of excreta, %				
week 2	14.2	14.9	15.1	13.1
3	13.9	16.5	15.6	14.9
4	15.5	15.2	12.9	13.2
5	16.2	15.9	14.7	14.9
6	18.3	19.3	18.2	17.3
mean ± SD	15.6 ± 1.76	16.4 ± 1.75	15.3 ± 1.91	14.7 ± 1.71
DM contents of litter, %				
week 2	55.9	58.8	69.2	59.2
3	42.8	45.3	58.1	49.1
4	37.3	38.4	44.9	40.4
5	34.7	39.2	41.9	39.0
6	38.4	47.7	41.4	46.6
mean ± SD	41.8 ± 8.39	45.8 ± 8.23	51.1 ± 12.2	46.8 ± 8.07
Final litter DM ² , %	44.8	46.6	46.5	47.9

¹ see Table 1; ² whole litter material (wood shavings, excreta, feathers) at the end of the experiment; SD – standard deviation

Table 5. Development of external foot pad scores of broilers fed diets with different protein sources and combinations

Food pad score	Treatment ¹			
	SBM	RSM	HBM	ALG
week 2	0.95 ± 0.508 ^a	1.25 ± 0.479 ^a	0.80 ± 0.410 ^a	0.60 ± 0.398 ^a
3	2.00 ± 0.934 ^a	2.95 ± 1.10 ^a	1.55 ± 0.564 ^b	2.15 ± 1.10 ^a
4	3.35 ± 0.98 ^b	4.20 ± 1.15 ^a	2.85 ± 1.21 ^c	3.90 ± 0.913 ^b
5	4.40 ± 1.02 ^b	5.25 ± 1.06 ^a	4.00 ± 1.22 ^c	4.90 ± 1.02 ^b
6	5.42 ± 1.21 ^b	6.28 ± 1.02 ^a	5.48 ± 1.54 ^b	6.58 ± 0.01 ^a

¹ see Table 1; ^{abc} – means within the same row with different superscripts are significantly different at $P < 0.05$

Discussion

In this study, parts of the soyabean meal (32.5% in group SBM) were replaced by rapeseed meal (RSM: 22.9% soyabean meal and 14.5% rapeseed meal), haemoglobin meal (HBM: 22.5% soyabean meal and 4.5% haemoglobin meal) or algae meal (ALG: 28.0% soyabean meal and 4.0% algae meal).

It is well known that feed intake depends not only on dietary energy content, but also on protein quality, which can be attributed to the perfect amino acid balance justifying the use of ideal protein concept (Nahashon et al., 2006; Tufarelli et al., 2007). The birds fed the RSM diet had the numerically highest absolute feed intake in this study. Nonetheless, this was not consistent with that reported by Kermanshahi and Abbasi Pour (2006) who found in a study on broiler chickens that adding 150 and 300 g · kg⁻¹ of RSM to diets had a negative effect on feed intake. However, Kölln et al. (2014) reported

that replacing SBM with up to 20% RSM led to a markedly higher feed intake in broilers (3 254 g vs 2 802 g). These contradictory results might be caused by the presence of different varieties of RSM and hence their qualities used in those studies (Zeb, 1998). In the ALG group, however, the feed intake was not different from that of the SBM group. Nevertheless, in the algae group there was a continuously higher water:feed intake ratio.

Regarding growth performance, no significant differences were found in the final BW between groups fed the SBM or ALG diets. It is well known that algae have a high protein quantity, these proteins being of high quality and comparable to conventional vegetable proteins (Becker, 2007). Similarly, in this study almost identical protein contents and amino acids levels were noted for both SBM and ALG diets. These results are also in agreement with those obtained by Evans et al. (2015) who concluded that diets containing 0, 6, 11 and 16% algae were statistically similar in performance to broilers fed SBM. However, at a higher inclusion rate of 21% algae diet there was a decrease in the performance and amino acid digestibility rate. The authors speculate that this may have been caused by the same process that increased firmness. Protein gelation during pelleting may have increased the viscosity of digesta, preventing endogenous enzymes gaining access to substrates in the digesta, thus decreasing performance and amino acid digestibility (Evans et al., 2015).

Kölln et al. (2014) found that replacing SBM with RSM up to 20% led to significantly higher BW in broilers (2 407 vs 2 106 g). It is an obvious relationship between feed intake and final body weight. Nevertheless, it was reported that including whole rapeseed and rapeseed meal at the 5, 10 or 15% levels linearly led to reduced performance (Toghyani et al., 2009). Commenting on these and similar discrepancies, two possible explanations are suggested: firstly, differences in the nutritional quality of the non-RSM part of the diet and secondly, differences in the chemical characteristics of the RSM used in the various experiments (March and Biely, 1971).

The significantly lower BW of the group fed the HBM diet in this study is supported by the findings of Khawaja et al. (2007) who reported that blood meal can be effectively used up to 3% only without having any adverse effects on broiler growth. Nevertheless, there are some reports indicating that adding 1 to 4% blood meal to diets can improve poultry performance (Donkoh et al., 2001).

FPD is an important aspect related to birds' welfare (Abd El-Wahab et al., 2011, 2012a). The present data show significant effects of different protein sources (despite the almost identical chemical composition of the four diets) on the FPD scores. However, it has to be pointed out that the high FPD scores might be related to the high water consumption caused by the unintended high dietary electrolyte content ($\text{Na } 2.0 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$, $\text{K } 8.9 \text{ g} \cdot \text{kg}^{-1} \text{ DM}$) and its effect on litter quality. The poorer results in the group RSM were unexpected, contrary to previous findings of Kölln et al. (2014) who found that replacing SBM with RSM up to 20% led to significantly lower FPD in broilers. These positive effects on foot pad health might be related to the reduced intake of stachyose, verbascose and raffinose (sticky excreta) by reducing the amount of SBM in the diets and consequently in the favourable DM contents of the litter (Kölln et al., 2014). In another study, different levels of dietary RSM up to $180 \text{ g} \cdot \text{kg}^{-1}$ diet had no significant effect on the severity of FPD in turkeys reared for 21 weeks (Mikulski et al., 2012).

Conclusions

Rapeseed meal could be the most suitable alternative protein source for broilers due to higher body weight gains. However, further studies are required to lower its effect on foot pad health. Although haemoglobin meal was the richest in protein, it is not recommended to use it more than 4% in the broiler diet. Feeding algae meal led to a comparable body weight gain to soyabean meal but its price might limit its use in poultry diets in addition to its adverse effects on foot pad health.

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