Effect of microalgae *Chlorella vulgaris* on laying hen performance

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SUMMARY

The components of green microalgae are believed to influence the bird reproduction and the egg quality. To study these effects, a feeding study with 182 laying hens (Lohmann Brown) at age of 22 weeks was accomplished. Seven groups with 26 hens per group were examined. Hens were kept individually in a cage battery until 8th laying month. The hens were artificially inseminated. The control basal diet was supplemented with 2.5 g, 5.0 g or 7.5 g of spray-dried (SD) or bullet-milled and spray-dried (BM-SD) *Chlorella vulgaris* per kg. Number of laid eggs was recorded daily and the feed consumption monthly. Each month the collected eggs were weighed four times within two weeks. In the 3rd and 6th laying month all eggs laid over 3 consecutive days were collected and the yolk weight, albumen weight, shell weight and yolk colour were analysed. 5-10 eggs per hen were collected over 10 days in the 2nd and 5th laying month and stored in the incubator for hatching. Two nitrogen (N)-balance studies (NbS) were performed. In the NbS1 36 laying hens (Lohmann Brown) were allocated to 3 groups, 12 hens each, and they were kept individually in metabolism cages. Control group was fed the basal diet and the experimental groups were offered the basal diet supplemented with either 5.0g SD or BM-SD *C. vulgaris* per kg. In the NbS2, 36 laying hens (Lohmann Selected Leghorn) were allocated to 4 groups, 9 hens each. They received the control diet supplemented with 2.5g, 5.0g, or 7.5g of BM-SD *C. vulgaris* per kg. In both experiments, the feed offered to the hens was controlled and was adjusted to 110g per hen in the adaptation period (5 days) and in the collection period (5 days), when the excreta were collected two times every day. In the feeding trial, the feed intake in the control was higher than in the 7.5g BM-SD. The laying intensity, egg weight, daily egg mass production and feed conversion were not affected. The yolk tended to be heavier and its quality was improved in the 5 and 7.5g SD and BM-SD, and in 2.5g BM in comparison to the control and the 2.5g SD group. The albumen weight was higher in the control and the 2.5g SD than in the other groups in the 2nd measure period. 7.5g SD improved egg shell weight in the 3rd and in the 6th laying month a tendency was observed. The yolk was more intensive in all SD and BM-SD groups. The hatching performance tended to be higher in the 5 and 7.5g SD and BM-SD, and 2.5g BM-SD. No differences in the N-balance were recorded.

Keywords: *Chlorella vulgaris*, green microalga, laying hen, laying performance, hatching performance

INTRODUCTION

Nutrition of laying hens focuses on sustaining health and the egg production with parallel improvement of egg quality. *Chlorella vulgaris*, a natural occurring green microalga has been artificially cultivated and is of even more interest for industry and nutrition (Pulz and Gross, 2004). Its components are believed to influence animals' performance and health, including the reproduction and the egg quality (Arakawa et al., 1960), however the results are equivocal and the nutritive value of open or indoor cultivated *C. vulgaris* depends upon the technological process used to treat the algal mass (Janczyk et al., 2006; 2007). Different thermal processes applied in order to destroy the robust cell wall restricting the enzymes' access to the cell components lead to denaturizing of amino acids and/or active substances of chlorella cells and affect the microalgal protein digestibility (Janczyk et al., 2007). In the present study, two differentially processed microalgal powders, i.e. spray-dried or bullet milled and then spray-dried were added to laying hen feed. The effect of such supplementation on laying hen performance was investigated in a longer-term feeding and in two short-term nitrogen-balance studies.

MATERIAL AND METHODS

Chlorella vulgaris

The unicellular green algae *C. vulgaris* (C1) were obtained from the Institute for Cereal Processing Ltd. (IGV), Nuthetal, Germany, where it had been cultivated in a closed photobioreactor PBR 4000 using sunlight (Pulz, 2001). Microalgal biomass harvested and used for the study was either spray dried in the compact spray dryer (Anhydro, Denmark) with delivery air temp. 300°C and exhaust air 105°C or it was bullet-milled with following spray drying.

Bullet-milled Chlorella vulgaris

The 10%-biomass of *C. vulgaris* was milled in the bullet mill (LME4, Netzsch Feinmahltechnik Ltd., Germany), containing glass bullets of size 0.5 – 0.7 mm, at 2,300 rpm for 18 min. The emptying of the system (20 L) lasted for 6 min and the microalgal biomass was spray-dried immediately thereafter.

Feeding study

A total of 182 laying hens (Lohmann Brown) were used in the feeding study. The hens were allocated to 7 groups, 26 hens each. The hens were kept individually in a cage battery. The control basal diet (Table 1) was supplemented with 2.5 g, 5.0 g or 7.5 g of spray-dried (SD) or bullet-milled and spray-dried (BM-SD) *Chlorella vulgaris* per kg. The experiment commenced when the hens were 22 weeks old and it continued until the 8th laying month. Each hen was offered the respective diet and water *ad libitum*. The hens were artificially inseminated. The number of laid eggs was recorded daily and the individual feed consumption was recorded monthly. The collected eggs were weighed four times in two weeks within each month. In the 3rd and 6th laying month all eggs laid over 3 consecutive days were collected and the yolk weight, albumen weight, shell weight and yolk colour were analysed. The colour of the egg yolk was measured with the Roche-Fan.

Table 1 Composition and chemical ingredients of the basal diet (g/kg)

Composition	Basal diet
Wheat	300.0
Corn	100.0
Lupin (*L. angustifolius*)	133.0
Pea	250.0
Wheat protein	88.5
Soya oil	22.2
Di-calcium-phosphat	11.0
Calcium carbonate	78.0
Sodium chloride	3.0
DL-methionine	2.0
L-lysine-HCl	2.0
Tryptophan	0.3
Premix [1)]	10.0
Dry matter [2)]	907/892/896
Crude protein [2)]	205/204/196
ME, MJ/kg [3)]	11.4
Lysine [3)]	8.2
Methionine + Cystine [3)]	5.9

[1)] vitamin- mineral premix provided per kg of diet: fe, 40 mg; cu, 10 mg; zn, 80 mg; mn, 100 mg; se, 0.25 mg; i, 1.2 mg; co, 0.55 mg; vitamin a, 10000 iu; vitamin d_3, 2500 iu; vitamin e, 20 mg; vitamin k_3, 4 mg; thiamine, 2.5 mg; riboflavin, 7 mg; pyridoxine, 4 mg; cobalamin, 20 µg; nicotinic acid, 40 mg; pantothenic acid, 10 mg; folic acid, 0.6 mg; biotin, 25 µg; choline chloride, 400 mg

[2)] analysed values, feeding trial/balance trial 1/balance trial 2

[3)] calculated values (WPSA; 1985)

Hatching study

Five to ten eggs per hen were collected over a period of 10 days in the 2nd and 5th laying months and they were stored in an incubator (Poultry hatchery, Ehret Labor- und Pharmatechnik GmbH & Co KG, Emmendingen, Germany).

The number of hatched chicks and their weight one day after hatching were recorded.

Nitrogen-balance studies

Two nitrogen (N)-balance studies (NbS) were performed. In the NbS1 36 laying hens (Lohmann Brown) were allocated to 3 groups, 12 hens each. The hens were kept individually in metabolism cages. Control group was fed the basal diet and the experimental groups were offered the basal diet supplemented with either 5.0 g SD or BM-SD *C. vulgaris*. In the NbS2, 36 laying hens (Lohmann Selected Leghorn - LSL) were allocated to 4 groups, 9 hens each. As in NbS1, the hens were kept individually and they received the basal diet (control) supplemented with 2.5 g, 5.0 g, or 7.5 g of BM-SD *C. vulgaris*. In both experiments, the feed offered to the hens was controlled and was adjusted to 110 g per hen in the adaptation period (5 days) and in the collection period (5 days). The hens were 24 weeks old when they entered the collection period, when the excreta were collected two times every day.

Statistical analysis

The data was subjected to analysis of variance (using the GLM procedure in the SAS operating system, Version 9.1, 2002/03). According to the standardized experimental conditions, it was not necessary to adjust for fixed effects of laying period, since they were meaningless. The focus was to compare the groups under study regarding their trait performances. The differences of mean between the groups were tested by several testing methods, as implemented in SAS. The results of various tests were similar with respect to significant differences. Here, the results of the Student-Newman-Keuls Test are presented. The differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Feeding study

The composition of the basal diet fed in all three studies is presented in Table 1. The composition of the SD *C. vulgaris* is summarized in Table 2. The analysed crude components of the SD *C. vulgaris* did not differ from the BM-SD *C. vulgaris,* which is similar to our previous observations, when neither electroporation nor ultrasonication affected the crude components of the indoor cultivated *C. vulgaris* (Janczyk et al., 2005). Also the supplementation of the basal diet with the microalgae had no impact on the analysed parameters of the experimental diets which remained the same as in the basal diet. When 10g/kg of *C. vulgaris* was added to a mice diet, also no effects of the microalgae on the diet components were observed (Janczyk et al., 2006). In this study we therefore confirmed the low amount of the microalgae has no effect on the diet composition.

Table 2. Chemical composition of spray-dried *Chlorella vulgaris* (units/fresh matter)[1]

Component	Amount	Units	Component	Amount	Units
Dry matter	96.94	g/100g	K	2260	mg/100 g
Ash	9.1	g/100g	Mg	388	mg/100 g
Protein (N x 6.25)	60.6	g/100g	Ca	581	mg/100 g
Fat	8.1	g/100g	Fe	172	mg/100 g
Fiber	20.8	g/100g	Cu	2.6	mg/100 g
Carbohydrates	5.6	g/100g	Zn	14.5	mg/100 g
Energy	1291	kJ/100 g	Mn	150	mg/kg
SFA[2]	25.1	g/100g	I	0.5	mg/kg
MSFA[2]	15.7	g/100g			
PSFA[2]	58.5	g/100g	Polyphenols (total)	0.4	g/100g

[1] spray-dried and bullet-milled-spray dried *Chlorella vulgaris* did not differ significantly in the composition. Therefore only the values for spray-dried *C. vulgaris* are presented.
[2]– MSFA, PSFA, SFA – (mono-), (poli-), saturated fatty acids

The performance parameters of the laying hens are presented in Table 3. The feed intake in the 7.5g BM-SD group was significantly lower than in the control. There was also a numerically lower feed intake in the 7.5g SD in comparison to the control group. Apparently, the higher level of the microalgae could have affected the taste of the feed. When we offered a feed supplemented with 10g/kg of microalgae to mice, we observed no effects on the daily feed intake (Janczyk et al., 2006). One can not exclude that birds react differently from mice on the presence of the microalgae in the feed. This would need to be investigated in a separate study, where the effect of the microalgae on the feed taste and/or the smell would have to be studied.

Table 3. Laying performance obtained from groups of laying hens fed a control diet supplemented with spray dried (SD) or bullet milled and spray dried (BM-SD) *Chlorella vulgaris* powder for 8 months (mean ± SD).

Trait	Control group	2.5g SD	5g SD	7.5g SD	2.5g BM-SD	5g BM-SD	7.5g BM-SD
Feed intake [g/hen/day]	122[a] ± 13	119[ab] ± 14	121[ab] ± 14	120[ab] ± 12	119[ab] ± 14	120[ab] ± 13	118[b] ± 12
Laying intensity [%]	95.8 ± 8	93.4 ± 11	95.0 ± 10	95.4 ± 9	93.9 ± 10	94.9 ± 9	94.4 ± 10
Egg weight [g/egg]	62.4 ± 6	62.7 ± 7	62.5 ± 6	62.8 ± 6	61.6±6	63.0 ± 6	61.3 ± 5
Egg mass production [g/hen/day]	59.9 ± 8	59.1 ± 10	59.7 ± 8	60.2 ± 8	58.3 ± 9	60.1 ± 8	58.3 ± 8
Feed conversion [kg/kg]	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.2	2.1 ± 0.3	2.0 ± 0.3	2.1 ± 0.3

a; b – Means with different letters differ significantly ($P \leq 0.05$)

The laying intensity of the hens remained at a high level of 93 to 96% throughout the 8 months of the study and did not differ among groups. The laying performance remained therefore at the maximal level over the whole study showing no adverse effects of the microalgae. As it was postulated, the supplementation of the bird diet with the microalgae would increase the seasonal laying performance (Arakawa et al., 1960). However, Arakawa et al. (1960) observed also no effect on the overall egg production. In opposite, in a field study an increase of the laying performance of hens was observed, however the hens used in that study were of low productivity (Janczyk, 2005). It is not possible to increase the maximum egg production, therefore in the present study no additional effect of adding *C. vulgaris* to the diet was observed.

The egg weights ranged between 62 and 63g in the 3rd month and 64-65g in the 6th month of laying, and they were not affected by the microalgae supplementation. As a consequence, the daily egg mass production and the feed conversion did not differ among groups. This finding is similar to the report of Arakawa et al. (1960), who observed no differences in the mean egg mass between control and microalgae group, with an average egg weight of 42g. In that study white Leghorns were used. The hybrids of Lohmann Brown hens used in the present study lay bigger eggs and the recorded egg mass was physiological for these hybrids. It is believed, the host welfare and productivity may be positively influenced by a higher diversity of the intestinal microbiota. The changes observed in this study may be therefore supported by our findings the cecal microbial population in the microalgal groups had higher diversity than in the controls (Janczyk et al., 2009).

Table 4. Quality traits of eggs collected in the 3rd and 6th laying month (LM) from groups of laying hens fed a control diet supplemented with spray dried (SD) or bullet milled and spray dried (BM-SD) *Chlorella vulgaris* powder (mean ± SD).

Trait	LM	Control goup	2.5g SD	5g SD	7.5g SD	2.5g BM-SD	5g BM-SD	7.5g BM-SD
Egg weight [g/egg]	3.	61.0 ± 5	61.5 ± 7	61.0 ± 5	62.3 ± 5	60.4 ± 5	62.9 ± 5	60.5 ± 4
	6.	64.7 ± 6	64.3 ± 6	64.6 ± 6	65.5 ± 6	63.9 ± 5	65.3 ± 5	63.1 ± 4
Yolk [g/egg]	3.	$15.9^{cd}\pm1$	$15.8^{d}\pm1$	$16.4^{abc}\pm1$	$16.5^{abc}\pm1$	$16.6^{ab}\pm2$	$16.9^{a}\pm1$	$16.2^{bcd}\pm1$
	6.	$16.4^{d}\pm2$	$16.2^{d}\pm1$	$18.6^{abc}\pm2$	$18.0^{c}\pm1$	$18.7^{ab}\pm2$	$19.1^{a}\pm2$	$18.2^{bc}\pm2$
Albumen [g/egg]	3.	38.0 ± 4	38.4 ± 5	37.6 ± 4	38.4 ± 4	36.8 ± 4	38.7 ± 4	37.3 ± 4
	6.	$40.9^{a}\pm5$	$40.5^{a}\pm5$	$38.5^{bc}\pm5$	$39.8^{ab}\pm5$	$37.5^{c}\pm4$	$38.6^{bc}\pm4$	$37.5^{c}\pm3$
Shell [g/egg]	3.	$7.1^{b}\pm0.5$	$7.3^{ab}\pm0.8$	$7.0^{b}\pm0.7$	$7.4^{a}\pm0.6$	$7.1^{b}\pm3.8$	$7.3^{ab}\pm0.6$	$7.0^{b}\pm0.6$
	6.	7.4 ± 0.6	7.6 ± 0.7	7.5 ± 0.7	7.7 ± 0.7	7.7 ± 0.6	7.6 ± 0.8	7.4 ± 0.8
Yolk colour[1)]	3.	$12.9^{d}\pm0.6$	$13.4^{b}\pm0.8$	$13.6^{b}\pm0.7$	$13.9^{a}\pm0.6$	$13.5^{b}\pm0.7$	$13.1^{c}\pm0.6$	$13.5^{b}\pm0.6$
	6.	$12.0^{b}\pm0.5$	$12.7^{a}+0.8$	$12.2^{b}\pm0.6$	$12.2^{b}\pm0.4$	$11.8^{c}+0.6$	$12.0^{b}\pm0.7$	$12.2^{b}\pm0.6$

a; b; c; d – Mean values with different letters differ significantly ($P\leq0.05$)

[1)]The intensity of the yolk colour was measured using the Roche-Fan pantone

Table 4 summarizes the egg quality recorded during the 3rd and 6th laying months. The albumen from eggs collected in the 6th month from the control and the 2.5g SD was heavier than that of the other groups. There was a tendency for the yolk to be heavier in groups 5 and 7.5g SD, where also the egg quality was in part significantly improved. This tendency was also recorded for the 5 and 7.5g BM-SD. Furthermore, the egg quality in the 2.5g BM-SD was improved compared to control group and the 2.5g SD. The eggs laid by the hens from all SD and BM-SD groups in the 3rd month had more intensive yolk colour than the eggs laid 3 month later. Even if not constant, the positive effect of the microalgae on the egg yolk quality was recorded. Arakawa et al. (1960) and Lipstein et al. (1980) also observed more intensive yellow colour of the yolk when the laying hens were fed diets supplemented with green microalgae, this was also a finding in the already mentioned field study (Janczyk, 2005). It was postulated, the carotenes and xantophylls from the microalgae influence the egg yolk colour (Arakawa et al., 1960). However, it remains unclear why the effect observed in the 3rd laying month was not observed in the 6th laying month.

Supplementation of the diet with 7.5g SD improved the egg shell weight in the 3rd month. However, in the 6th laying month no significant differences in the shell weight were observed, and the shells from the microalgal groups were only numerically heavier than the ones from the control eggs. This observation remains in agreement with the report of Arakawa et al. (1960) and indicates a better calcification of the shell. However, the underlying mechanisms still remains unknown.

Table 5. Egg weight, one-day chicken weight and hatched living chicks of fertilized eggs collected in the 2nd and 5th laying month (LM) from groups of laying hens fed a control diet supplemented with spray dried (SD) or bullet milled and spray dried (BM-SD) *Chlorella vulgaris* powder for 8 months (mean ±SD)

Trait	LM	Control group	2.5g SD	5g SD	7.5g SD	2.5g BM-SD	5g BM-SD	7.5g BM-SD
Egg weight [g/egg]	2.	58.9±1	58.6±5	56.4±5	58.6±5	59.4±4	60.0±4	58.6±3
	5.	64.7±6	65.9±7	65.2±6	64.4±5	64.7±4	64.6±5	63.5±4
Chicken weight [g/chick]	2.	40.5±5	39.4±3	40.3±3	40.5±4	40.4±3	39.8±6	41.2±6
	5.	44.1±4	44.7±5	44.2±5	43.0±5	43.7±3	43.2±3	41.6±7
Hatched living chicks [%]	2.	66.8±25	67.4±25	74.1±24	72.6±25	77.4±26	78.9±19	75.5±24
	5.	88.4±19	87.7±18	88.2±20	91.4±12	93.4±13	81.4±21	91.2±13

Hatching study

The results of the two hatching trials are listed in Table 5. In the 2nd month of laying, the proportional share of hatched living chicks in the 5 and 7.5g SD and BM-SD, as well as in the 2.5g BM-SD tended to be higher compared to the control and to the 2.5g SD. This observation could not be confirmed in the 5th laying month. The effect of the microalgae on the reproduction with resulting increased number of rat and mice pups was observed (Ishibashi, 1971; Kapoor & Mehta, 1993) but could not always be confirmed (Fevrier & Seve 1975;

Janczyk et al., 2006). The authors found no data regarding the effects of green microalgae on hatching performance in hens. This is the first report indicating that *Chlorella vulgaris* might influence the number of hatched chicks positively.

Table 6. Results of the N-balance studies obtained from groups of laying hens fed a control diet supplemented with spray dried (SD) or bullet milled and spray dried (BM-SD) *Chlorella vulgaris* powder for 10 days (mean ±SD)

Trait	Study	Control group	2.5g SD	5g SD	7.5g SD	2.5g BM-SD	5g BM-SD	7.5g BM-SD
N-balance	1	5.7±0.7	-	6.6±1.4	-	-	6.5±0.6	-
[g N/hen] [1)]	2	9.4±0.7	-	-	-	9.3±0.8	9.3±0.8	9.3±0.9
PPV [%] [2)]	1	42.1±3.9	-	44.9±8.2	-	-	45.6±4.3	-
	2	61.2±4.6	-	-	-	62.0±0.3	60.0±5.3	61.3±3.7

[1)] N-balance=N-intake – N-excreta (faeces-N + urine-N)
[2)] Productive protein value (PPV) = (N-balance: N-intake) x 100

N-balance studies

The N-balance and the productive protein value calculated in the two studies are summarized in Table 6. There were no differences among groups regarding the protein accretion in either study. However, the N-balance and PPV were lower in the NbS1, when the Lohmann Brown hens were used, in comparison to the NbS2, when the Lohmann Selected Leghorns were taken for the study. In the NbS2 only BM-SD *C. vulgaris* was investigated, as the protein of this microalgal powder was shown to have higher digestibility than the SD *C. vulgaris* (Janczyk et al., unpublished). The low amount (up to 7.5g/kg in this study) of *C. vulgaris* powder had no negative effect on the feed protein value, an observation that confirmed the results recorded in a mice study (Janczyk et al., 2006).

CONCLUSIONS

In the present study we have shown a supplementation of a hen diet with the green microalgae *Chlorella vulgaris* positively affected the laying performance, increasing the number and quality of laid eggs, as well as the hatching performance. However, this phenomenon seems to be pronounced at the beginning of the laying period. No adverse effects of the microalgae on the hen health or protein accretion were observed. Spray dried or bullet milled and spray dried *Chlorella vulgaris* can therefore be used as a dietary supplement for laying hens.

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