



# Energy values and chemical composition of spirulina (*Spirulina platensis*) evaluated with broilers<sup>1</sup>

Renata Ribeiro Alvarenga<sup>2</sup>, Paulo Borges Rodrigues<sup>2</sup>, Vinícius de Souza Cantarelli<sup>2</sup>, Márcio Gilberto Zangeronimo<sup>3</sup>, José Walter da Silva Júnior<sup>4</sup>, Leonardo Rafael da Silva<sup>2</sup>, Luziane Moreira dos Santos<sup>2</sup>, Luciano José Pereira<sup>3</sup>

<sup>1</sup> Financiado pelo CNPq.

<sup>2</sup> Departamento de Zootecnia, Universidade Federal de Lavras.

<sup>3</sup> Departamento de Medicina Veterinária, UFLA.

<sup>4</sup> Ouro Fino Agronegócio.

**ABSTRACT** - The objective of this study was to determine the chemical and energy composition of spirulina (*Spirulina platensis*), the nutrient metabolizability coefficients, and the values of apparent metabolizable energy (AME) and the apparent metabolizable energy corrected for nitrogen balance (AMEn) in broilers. A digestibility trial was carried out by using total excreta collection method, with 90 Cobb 500 lineage chicks, with initial weight of  $256 \pm 5$  g at 11 days of age. Birds were allotted in metabolic cages for 10 days, distributed in a completely randomized design, with three treatments and six repetitions with five birds each. Diets consisted on a reference-ration based on corn and soybean meal and two test diets, one containing spirulina (30%) and the other one with soybean meal (30%). Spiruline was superior to soybean meal for contents of dry matter (DM), gross energy (9.60%), crude protein (26.56%), ether extract (54.45%), mineral matter (42.77%), calcium (100%) and total phosphorus (130.77%) and also for most amino acids, except lysine, glutamate, histidine and proline. Nevertheless, spiruline presented lower values of gross fiber (83.95%), acid detergent fiber (85.12%) and neutral detergent fiber (6.15). The AME and AMEn values (kcal/kg of DM) were, respectively, 2,906 and 2,502 for the spirulina and 2,646 and 2,340 for the soybean meal and AMEn of spirulina was 6.92% higher than soybean meal.

Key Words: non-conventional food, nutritional value, total collection

# Valores energéticos e composição química da espirulina (Spirulina platensis) avaliada com frangos de corte

**RESUMO** - Objetivou-se determinar a composição química e energética da espirulina (*Spirulina platensis*), os coeficientes de metabolizabilidade dos nutrientes e os valores de energia metabolizável aparente (EMA) e aparente corrigida pelo balanço de nitrogênio (EMAn) em frangos de corte. Realizou-se um ensaio de digestibilidade utilizando-se a metodologia de coleta total de excretas com 90 pintos machos da linhagem Cobb 500, com peso inicial de  $256 \pm 5$  g aos 11 dias de idade. As aves foram alojadas em gaiolas de metabolismo durante dez dias, distribuídas em delineamento inteiramente casualizado, com três tratamentos e seis repetições, cada uma com cinco aves. As dietas consistiram de uma ração-referência formulada à base de milho e farelo de soja e duas rações-teste, uma contendo espirulina (30%) e outra com farelo de soja (30%). A espirulina foi superior ao farelo de soja quanto aos conteúdos de matéria seca, energia bruta (9,60%), proteína bruta (26,56%), extrato etéreo (54,45%), matéria mineral (42,77%), cálcio (100%) e fósforo total (130,77%) e também para a maioria dos aminoácidos, exceto lisina, glutamato, histidina e prolina. No entanto, apresentou menores valores de fibra bruta (83,95%), fibra em detergente ácido (85,12%) e fibra em detergente neutro (6,15%). Os valores de EMA e EMAn (kcal/kg de MS) foram, respectivamente, 2.906 e 2.502 para a espirulina e 2.646 e 2.340 para o farelo de soja, e a EMAn da espirulina foi 6,92% superior à do farelo de soja.

Palavras-chave: alimento não-convencional, coleta total, valor nutricional

#### Introduction

The Brazilian poultry industry has been in evidence in the international market because of the high productivity and quality of its products. However, to maintain this profile, it is important to develop nutritional strategies and to formulate adequate diets to birds, increasing the profitability of the producer.

Corn and soybean meal are the main components of the diets for broilers, which represent approximately 75% of production costs. Considering the large economic fluctuations of these ingredients in the market, it becomes

necessary to search for alternative food to reduce production costs. However, in order to construct a precise formulation that meets the nutritional requirements of animals, it is necessary to know the characteristics of these ingredients, such as chemical and energy composition.

Thus, the production of microalgae has received special attention recently, because, according to Rogatto et al. (2004), these microorganisms can be a good alternative source of protein in the diet. Among several microorganisms which have been studied, the blue-green alga spirulina is considered a promising microorganism due to its high protein content (65 to 70% DM) and great amount of vitamins and minerals (Contreras et al. 1979; Kay, 1991). Moreover, with the high reproduction rate of spiruline, it is estimated that some area available for their growth can produce 125 times more protein than the same area of corn (Furst, 1978).

*Spirulina platensis* is a microalgae with appropriate composition to be used as a food supplement. It is commonly used by humans and animals as protein source. Several studies such as palatability, lack of toxicity and easy digestion, antioxidant actions, hypocholesterolemic, anticancer, immunostimulant, anti-inflamatory, antiviral, among others have been conducted to verify the possible benefits of spirulina and some properties have been verified (Rodriguez-Hernández et al., 2001; Derne et al., 2006; Colla et al., 2007).

For animal feed, according to Ross & Dominy (1990), the addition of 1.5 to 12% of spirulina into the diets for broilers can replace other protein sources, especially soybean meal, with satisfactory growth rates and feed efficiency. However, the optimal levels for using this alga as a substitute source of the conventional protein on a diet are still controversial (Soler et al. 2000; Lacaz-Ruiz,2003; Becker,2007). Thus, new studies are necessary in order to evaluate the nutritional value of spirulina for these animals.

The objective of this study was to determine the chemical and energy composition, the apparent metabolizable energy (AME) and AME corrected for nitrogen balance (AMEn) of spirulina and to compare those variables to soybean meal as a possible replacement in diets for broilers.

## **Material and Methods**

The experiment was conducted in the Setor de Avicultura in the Departamento de Zootecnia at Universidade Federal de Lavras (UFLA), Lavras, Minas Gerais, Brazil.

It was used 90 broilers, Cobb 500®, with initial weight of 256 ± 5g from 11 to 21 days of age. The animals were

housed in groups of five birds per metabolic cage  $(50 \times 50 \times 50 \text{ cm})$ , equipped with trough shaped feeder, pressure drinker and aluminum tray. The environment was partially controlled by a digital device and artificial light for 24 hours. The experimental period was ten days, seven days for adaptation to the cages and food, and three days for total fecal collection (Sibbald & Slinger, 1963, adapted by Rodrigues et al., 2005).

Spirulina and soybean meal were evaluated by replacing the reference diet at 30%, based on fresh matter (Matterson et al., 1965). The reference diet was based on corn and soybean meal (Table 1). The metabolizable energy of the reference-diet and diets containing each feed to be evaluated were determined in six replicates of five broilers per experimental unit.

The experimental diets were provided *ad libitum* during the period that the broilers remained in their cages. Feeders were supplied three times a day, to avoid waste. The excreta collection was performed once a day, always starting at 8:00 a.m..

During excreta collection (birds at 19 to 21 days of age), the trays were previously coated with plastic to prevent excreta loss. Diets and leftovers were weighed and recorded, respectively, at the beginning and the end of the experimental

Table 1 - Centesimal composition and calculated values of the reference diet

Ingredient	Reference diet
Corn	60.98
Soybean meal	33.40
Soybean oil	2.00
Dicalcium phosphate	1.80
Limestone	0.85
Salt	0.40
DL-methionine 98%	0.25
L-lysine 78%	0.20
Vitamin supplement <sup>1</sup>	0.05
Mineral supplement <sup>2</sup>	0.05
Anticoccidial <sup>3</sup>	0.02
Calculated composition*	
Crude protein(%)	20.2
Metabolizable energy (kcal/kg)	2,991
Available phosphorus(%)	0.44
Calcium (%)	0.88
Digestible lysine <sup>4</sup> (%)	1.13
Digestible methionine <sup>4</sup> (%)	0.54
Methionine + digestible cystine <sup>4</sup> (%)	0.82
Digestible threonine <sup>4</sup> (%)	0.69

 $<sup>^1</sup>$  Vitamin supplement containing, per kg of product: Vitamin A – 12,000,000 IU; vitamin D<sub>3</sub> – 2,200,000 IU; vitamin E – 30,000 IU; vitamin B<sub>1</sub> – 2.2 g; vitamin B<sub>2</sub> – 6.0 g; vitamin B<sub>6</sub> – 3.3 g; vitamin B<sub>12</sub> – 0.016g; nicotinic acid – 53.0 g; pantothenic acid – 13.0 g; vitamin K<sub>3</sub> – 2.5 g; folic acid – 1.0 g; antioxidant 120.0 g – vehicle q.s.p – 1,000 g.

\* Calculated composition according Rostagno et al. (2005).

<sup>&</sup>lt;sup>2</sup> Mineral supplement containing, per kg of product: Manganese - 75.0 g; iron - 20.0 g; zinc - 50.0 g; copper - 4.0 g; cobalt - 0.2 g; iodine - 1.5 g; selenium - 0.25 g - vehicle q.s.p 1,000 g.

<sup>&</sup>lt;sup>3</sup> Salinomycin 12%.

<sup>&</sup>lt;sup>4</sup> Values of amino acids expressed in true ileal digestibility.

period for determining the consumption of each plot during this phase. The collected excreta was packed in previously identified plastic bags and stored in a freezer at -5°C until the end of the total collection period. Then, it was thawed, weighed and homogenized.

Afterwards, 400-g aliquots were removed, then sent to Laboratório de Nutrição Animal at the Universidade Federal de Lavras. These samples were submited to a pre-drying process in forced ventilation air (55°C) for 72 hours. Subsequently, they were re-weighed for determination of the dry matter and grounded into a mill, with 1.0- mm sieve and, then, dry matter, gross energy and nitrogen were determined. A sample of the reference diet was treated in the same manner, using standard techniques described by Silva (2002), adapted from AOAC (1995). Based on results obtained in laboratory, it was calculated the apparent metabolizable energy (AME) by using the equations proposed by Matterson et al. (1995) and adjusted for the nitrogen retention:

FT or RD AMEn= $\underline{GE \text{ intake} - (GE \text{ excreted} + 8.22*NB)}$ DM intake

feed AMEn = AMEnRD +  $\underline{AMEnFT} - \underline{AMEnRD}$ g/g of replacement

where: FT = feed test; RD = reference diet; GE = gross energy; NB = nitrogen balance (N consumed – N excreted); DM = dry matter.

For each diet, the gross energy, dry matter, crude protein, nitrogen, ether extract, gross fiber, acid and neutral detergent fiber, mineral matter, calcium and phosphorus were determined according to the techniques described by AOAC (1990) adapted by Silva & Queiroz (2002). The determination of amino acids was accomplished by using high performance liquid chromatography (HPLC). Values of gross energy of feed, excreta and food were determined in a calorimetric pump, model Parr-1261 and nitrogen by the Kjeldahl method.

It was determined the chemical composition, the energy (gross energy, metabolizable energy and apparent metabolizable energy corrected for nitrogen) and metabolizable coefficients of dry matter, crude protein, calcium and phosphorus of spirulina and soybean meal by using the methods described by Sakomura & Rostagno (2007).

# **Results and Discussion**

Numerically, comparing the nutritional values found for spirulina and soybean meal (Table 2) used in this study, it appears that spirulina showed similar quantities of dry matter. However, the energy and chemical compositions were completely different.

Compared to soybean meal, spirulina showed greater values of dry matter, gross energy (9.60%), crude protein (26.56%), ether extract (54.45%), mineral matter (42.77%), calcium (100%) and total phosphorus (130.77%), but lower crude fiber (83.95%), acid detergent fiber (85.12%) and neutral detergent fiber (6.15%). These observed differences, particularly the fiber, were caused by the levels of bark present in soybean meal. On the other hand, the high levels of calcium and phosphorus found in spirulina were caused by the differentiated cellular metabolism inasmuch as these elements are actively involved in the metabolism of microalgae.

Ross and Dominy (1990) analyzed the composition of *Spirulina platensis* and found similar values of crude protein (60.5%) and phosphorus (0.95%). However, the authors found a greater quantity of dry matter (94.5%) and lower calcium (0.28%) with regard to those obtained in the present work. According to another study compiled by Becker (2007), the value of crude protein in dry matter of the spirulina can vary from 46 to 63% and ether extract from

Table 2 - Chemical and energy composition of spirulina (Spirulina platense	<i>vis</i> ) and soybean meal, expressed on natural matter basis <sup>1</sup>
--	---

Composition	Spiruline	Soybean Meal
Dry matter (%)	88.08	89.01
Crude protein (%)	58.20	46.47
Gross energy (kcal/kg)	4,286	3,952
Ethereal extract (%)	2.60	1.70
Crude fiber (%)	0.78	4.91
ADF (%)	0.79	5.36
NDF (%)	10.61	11.42
Mineral matter (%)	8.44	5.97
Calcium (%)	0.48	0.24
Total phosphorus (%)	1.06	0.46
Apparent metabolizable energy (kcal/kg)	2,560	2,355
Apparent metabolizable energy corrected by nitrogen balance (kcal/kg)	2,204	2,083

<sup>1</sup> Analyses performed at the laboratory of Animal Nutrition of the DZO/UFLA.

4.0 to 9.0%, depending on the cultivation system of these algae. In the present work, the lipid content was lower than stipulated by these authors.

In respect to energy values, spirulina showed higher gross energy (9.6%), higher apparent metabolizable energy (9.8%) and, consequently, higher apparent metabolizable energy corrected for nitrogen (6.9%)compared to soybean meal.

Under conditions of ad libitum feeding, apparent metabolizable energy (AME) is higher than apparent metabolizable energy corrected for nitrogen (AMEn) when the nitrogen retention is positive (Wolynetz & Sibbald, 1984). This was the case of this experiment, in which broilers had unlimited consumption. However, it is important to emphasize that differences obtained among these values are associated with the nutrients digestibility, which in turn is influenced by the intake, the fiber content present on a diet, genetics of those broilers and environmental conditions that are directly linked to the ability of nitrogen retention by animals. This becomes evident when analyzing the results obtained by Rodrigues et al. (2002), where the apparent metabolizable energy exceeded apparent metabolizable energy corrected for nitrogen at 164 kcal/kg of dry matter when used in growing chicks, but it was lower than 226 kcal/kg when used in roosters.

In this study, it was observed that the apparent metabolizable energy was 404 kcal/kg higher in dry matter for spirulina and 306 kcal/kg for soybean meal. Rostagno et al. (2005) reported for soybean meal values of 2,256 kcal/kg to metabolizable energy.

Analysis of the amino acid composition of those studied foods showed that spirulina presented higher quantity of amino acids, lower quantity only for glutamato, histidine and proline and similar quantity of aspartic acid and lysine (Table 3). Likewise, spirulina showed higher amino acid/lysine for most of those nutrients, except for histidine, glutamate and proline. As lysine levels were similar, these results suggest higher protein levels for spirulina with regard to soybean meal.

Comparing the quantity of amino acids, it appears that these values are lower than the crude protein obtained in the same food. This fact is justified by the amount of nonprotein nitrogen in food. According to Becker (2007), studies have shown that non-protein nitrogen in spirulina can reach 11.5%, represented by the nucleic acids, glucosamine, nitrogenous substances in the cell wall and other amines. Thus, the calculation of crude protein can usually be overestimated when considering this food.

As demonstrated before, spirulina had greater sulfur amino acid content when compared to soybean meal, overcoming even the bran corn gluten 60, which, according to Rostagno et al. (2005), presents 1.39% of methionine and 2.46% of methionine and cystine. Considering the ideal protein concept in the diet formulation, the use of spirulina as a substitute for soybean meal allows less inclusion of methionine in its synthetic form. However, it is important to consider that proteins are composed of different amino acids and their quality is not only related to the amount of amino acids, but also to the relationship between them and the availability of each one in the gastrointestinal tract.

 Table 3 - Amino acid composition and relationship between amino acid/lysine of spirulina (Spirulina platensis) and soybean meal used in diets for broilers

Amino acid	Amino acid composition (%) <sup>1</sup>		Amino acid value/lysine	
	Spiruline	Soybean meal	Spiruline	Soybean meal
Aspartate	5.34	5.29	196	189
Glutamate	8.15	8.65	300	309
Serine	2.92	2.42	107	86
Glycine	3.00	2.01	110	72
Histidine	1.00	1.38	37	49
Arginine	3.96	3.55	146	127
Threonine	2.84	1.85	104	66
Alanine	4.54	2.02	167	72
Proline	2.15	2.36	79	84
Tyrosine	2.58	1.74	95	62
Valine	3.34	2.03	123	73
Methionine	1.98	0.79	73	28
Cystine	0.72	0.59	26	21
Isoleucine	3.06	2.04	113	73
Leucine	4.84	3.40	178	121
Phenylalanine	2.50	2.29	92	82
Lysine	2.72	2.80	100	100
Total	55.65	45.20	-	-

<sup>1</sup> Analyses performed at the CBO Laboratorial Analyses Campinas, SP.

With regard to metabolizable coefficients of crude protein, the results were in agreement with those obtained by Becker (2007), which showed values of 52.7% (Table 4). According to the literature, these values may vary from 51 to 63% (Ciferri, 1982; Tiboni & Ciferri, 1985). Considering the other nutrients and energy, metabolizable coefficients were very satisfactory, showing that spirulina can be used as a potential protein source for poultry.

Spirulina has a relatively high content of protein, and contain all essential amino acids. Studies in literature showed that the metabolizable energy can vary from 2,500 to 3,290 kcal/kg and phosphorus can reach 41% (Blum et al., 1976; Yoshida & Hoshi, 1980). In addition, spirulina is rich in thiamine, riboflavin, pyridoxine, cobalamin, ascorbic acid and antoxidant carotenoids (Ross & Dominy, 1990) and may be a good alternative to replace soybean meal in diets for broiler chickens.

 Table 4 - Coefficients of metabolizable energy and of some nutrients from spirulina (Spirulina platensis) obtained with broilers

Component	Coefficient of metabolizable (%)	Deviation
Dry matter	62.97	± 2.96
Crude protein	52.86	$\pm 4.58$
Gross energy	56.11	$\pm 2.04$
Phosphorus	74.32	± 5.53
Calcium	64.07	± 7.87

## Conclusions

Composition of spirulina shows higher values than those determined for soybean meal, except for crude fiber and for neutral and acid detergent fiber. The values of AME and AMEn (kcal/kg of DM) are, respectively, 2,906 and 2,502 for spirulina and 2,646 and 2,340 for soybean meal.

#### Acknowledgements

The authors gratefully acknowledge the support of Ouro Fino Agronegócio in this work and the INCT-CA (Instituto Nacional de Ciência e Tecnologia – Ciência Animal).

# References

- ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTRY -AOAC. Official methods of analysis. 16.ed. Arlington: AOAC International, 1995. 1025p.
- BECKER, B.W. Micro-algae as a source of protein. Biotechnology Advances, v.25, n.2, p.207-210, 2007.

- BLUM, J.C.; GUILLAUMIN, S.; CALET, C. Valeur alimentaire des algues Spirulines pour la poule pondeusse. Annales de la Nutrition et de l'Alimentaire, v.30, p.675-682, 1976.
- CIFERRI, O. Spirulina, the edible microorganism. Microbiology Review, v.47, n.4, p.551-578, 1982.
- CIFERRI, O.; TIBONI, O. The biochemistry and industrial potential of Spirulina. Annual Reviews of Microbiology, v.39, p.503-526, 1985.
- COLLA, L.M.; FURLONG, E.B.; COSTA, J.A.V. Antioxidant properties of Spirulina (*Arthrospira platensis*) cultivated under different temperatures and nitrogen regimes. **Brazilian Archives** of Biology and Technology, v.50, n.1, p.161-167, 2007.
- CONTRERAS, A.; HERBERT, D.C.; GRUBBS, B.G. et al. Bluegreen alga, spirulina, as the sole dietary source of protein in sexually maturing rats. **Nutrition Reports International**, v.19, n.6, p.749-63, 1979.
- DERNER, R.B.; OHSE, S.; VILLELA, M. et al. Microalgas, produtos e aplicações. Ciência Rural, v.36, n.6, p.1959-1967, 2006.
- FURST, P.T. Spirulina a nutricious alga, once a staple of Aztec diet, could feed many of the world hungry people. Human Nature, v.3, p.60, 1978.
- KAY, R.A. Microalgae as food and supplement. Food Science and Nutrition, v.30, n.6, p. 555-573, 1991.
- LACAZ-RUIZ, R. Espirulina: estudos e trabalhos. São Paulo: Roca, 2003. 296p.
- MATTERSON, L.D.; POTTER, L.M.; STUTZ, M.W. The metabolizable energy of feed ingredients for chickens. Agricultural Experimental Station Research Report, v.7, p.3-11, 1965.
- RODRIGUES, P.B.; ROSTAGNO, H.S.; ALBINO, L.F.T. et al. Valores energéticos da soja e subprodutos da soja, determinados com frangos de corte e galos adultos. **Revista Brasileira de Zootecnia**, v.31, n.4, p.1771-1782, 2002.
- RODRIGUES, P.B.; MARTINEZ, R.S.; FREITAS, R.T.F. et al. Influência do tempo de coleta e metodologias sobre a digestibilidade e o valor energético de rações para aves. Revista Brasileira de Zootecnia, v.34, n.3, p.882-889, 2005.
- RODRIGUEZ-HERNANDEZ, A.; BLÉ-CASTILLO, J.L.; JUÁREZ-OROPEZA, M.A. et al. *Spirulina maxima* prevents fatty liver formation in CD-1 male and female mice with experimental diabetes. Life Sciences, v.69, n.9, p.1029-37, 2001.
- ROGATTO, G.P.; OLIVEIRA, C.A.M.; SANTOS, J.W. et al. Influência da ingestão de espirulina sobre o metabolismo de ratos exercitados. **Revista Brasileira de Medicina do Esporte**, v.10, n.4, p.258-263, 2004.
- ROSS, E.; DOMINY, W. The nutritional value of dehydrated, bluegreen algae (*Spirulina platensis*) for poultry. **Poultry Science**, v.69, n.5, p.794-800, 1990.
- ROSTAGNO, H.S.; ALBINO, L.F.T.; DONZELE, J.L. et al. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 2.ed. Viçosa, MG: UFV, 2005. 186p.
- SAKOMURA, N.K.; ROSTAGNO, H.S. Métodos de pesquisa em nutrição de monogástricos. Jaboticabal: Funep, 2007. 283p.
- SIBBALD, I.R.; SLINGER, S.J. A biological assay for metabolizable energy in poultry feed ingredients together with findings which demonstrate some of the problems associated with evaluation of fats. **Poultry Science**, v.42, n.1, p.13-25, 1963.
- SILVA, D.J.; QUEIROZ, A.C. Análise de alimentos: métodos químicos e biológicos. 3.ed. Viçosa, MG: UFV, 2002. 235p.
- SOLER, A.; VALDIVIÉ, M.; DIEPPA, O. Uso de la Spirulina como pigmentante de la piel y la grasa de los pollos de ceba. Revista de la Facultad de Ciencias Veterinárias, v.41, n.1-3, p.19-24, 2000.
- WOLYNETZ, M.N.; SIBBALD, I.R. Relationships between apparent na true metabolizable energy and the effects of a nitrogen correction. **Poultry Science**, v.63, n.7, p.1386-1399, 1984.
- YOSHIDA, M.; HOSHI, H. Nutritive value of spirulina, green algae, for poultry feed. Japan Poultry Science, v.17, p.27-30, 1980.