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Brewery waste as a substitute for soy protein in soy-brewer's yeast mixtures to feed broiler chickens

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SUMMARY. We examined the replacement of soy isolated protein by a solid fraction coming from brewery liquid waste, in the preparation of soy protein and brewer's yeast mixtures (50:50) to feed growing chickens. The replacement of 20 percent soy protein by brewery waste protein to the diet, showed no significant differences in the growth and food intake of the chickens, when compared with soy protein fed chickens. Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) values of the diets were also very similar and the concentration of plasma and liver lipids remained approximately the same. Higher levels of brewery waste reduced the performance of chickens although total lipids, cholesterol and triacylglycerols in plasma, as well as total lipids and cholesterol in liver were not affected. The data reported here indicated that brewery waste can be used as a complementary protein source in broiler chicken diets.

RESUMEN. Uso del desecho de cervecería (jugo de nepe) en la sustitución de la proteína de soya en dietas para aves. Se investigó la sustitución del aislado proteico de soya por sólidos del jugo de nepe de cervecería, en dietas para pollos basadas en mezclas de proteína de soya y levadura de cerveza. La inclusión de 20 % de jugo de nepe seco a expensas de proteína de soya, no produjo diferencias significativas en el crecimiento, consumo de alimento, índice de eficiencia proteica (PER), utilización proteica neta (NPR), ni en las concentraciones de los lípidos plasmáticos y hepáticos. Sin embargo, niveles superiores de jugo de nepe afectaron la utilización de la proteína dietaria, si bien los lípidos plasmáticos y hepáticos no fueron modificados. Los resultados indicaron que el jugo de nepe seco puede usarse como proteína complementaria en dietas para pollos en crecimiento.

INTRODUCTION

For economical reasons, it is very important for developing countries to replace imported protein sources for example soy bean protein, in poultry diets. Agricultural and food industry wastes are an economical source of ingredients for feed, because it can be partly converted by animal in meat or eggs.

Previously we determined that the brewer's yeast used in the beer industry, could replace up to 50 percent of soy protein in broiler chicken diets (1). Nevertheless it is still necessary to find another complementary protein to replace the rest of the soy protein in the diet.

Brewery liquid waste is the resulting residue from brewing of beer and it constitutes a major industrial waste in Venezuela. It contains 20 percent of insoluble material and high carbohydrate levels and it has been shown that the clarified and hydrolysated waste is useful as a substrate for producing biomass (2), vitamins (3) and enzymes (4). However, the dry insoluble fraction contains nearly 55 percent of protein, this is higher than cereals and similar to some commercially available protein concentrates, constituting a plausible and economical source of protein for animal feed.

The present study attempted to evaluate the possibility of using dry solids of brewery waste as a complementary protein source in broiler chicken.

Since the effects of dietary proteins on lipids metabolism have been reported by many investigators (5), the plasma and liver lipids were also measured.

MATERIALS AND METHODS

Protein sources: The liquid brewery waste was collected for several days of production from a local plant effluent (Industrias Polar, Caracas) and was kept at 4 °C for a maximum time of two days. It was centrifugated for 5 minutes at 4080 xg in a refrigerated Sorvall centrifuge using a GSA rotor. The pellets were pooled and dried at 60 °C for 24 hours and then ground in a manual mill. Brewer's yeast was collected as flakes from rolling dryers and was obtained from the same plant. Soy-bean protein was isolated soy protein, 90 percent crude protein (Ralston, Purina. St. Louis MO).

Chemical analysis: The standard methods of proximate analysis (6) were used to determine dry matter, ether extract, ash and crude fiber. Nitrogen was estimated by a colorimetric determination of ammonia (7) and crude protein was calculated multiplying the nitrogen concentration by 6.25 (Nx6.25). The amino acid composition of the brewery waste was determined using an amino acid auto analyzer (Multichrom-Beckman 4225) from samples hydrolyzed in 6N HCl (8); available lysine was determined by the trinitrobenzenesulfonic acid method (9) and the brute energy content was determined by bomb calorimetry.

Growth assay: Forty nine, one-day-old, Warren chickens purchased in a local hatchery, were conditioned as described previously (1). After the conditioning period, the chickens were assigned by

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selective randomization to one of seven different groups, seven animals per group.

The basal diet and the vitamin and mineral mixtures were similar in composition to that described previously (10).

Table 1 shows the composition of the diets. Soy protein was the protein control (diet 1). In diets 2-5, brewer's yeast provided 50 percent of the protein and they were supplemented with a free thiamin and niacin vitamin mix in order to correct the brewer's yeast contribution (Millán N, unpublished). In diet 6 brewery waste was the sole protein source and diet 7 was a protein free diet.

The duration of the trial was 22 days. Feed and water were supplied *ad libitum*. A record of consumed was done daily and weight gain every other day. The protein efficiency ratio (PER), the net protein ratio (NPR) and the feed efficiency of the diets were calculated from growth and feed consumption data.

TABLE 1
Composition of the diets

	Diet Protein Mixture (%)						
	1	2	3	4	5	6	7
S	100	50	30	10	0	0	0
BY	0	50	50	50	50	0	0
BW	0	0	20	40	50	100	0
Ingredient (g per 100 g)							
Soybean protein	27.3	11.8	7.1	2.4	-	-	-
Brewer's yeast	-	25.0	25.0	25.0	25.0	25.0	-
Dried brewery waste	-	-	7.8	15.0	19.4	38.9	-
Corn starch	62.8	49.7	47.7	45.6	44.5	51.8	86.5
Corn oil	4.0	4.0	3.1	2.1	1.7	-	4.0
dl-methionine	0.46	0.46	0.46	0.46	0.46	0.46	0.46
Dicalcium phosphate	3.5	3.4	3.5	3.5	3.5	3.5	3.5
Mineral mix[a]	3.5	3.4	3.5	3.5	3.5	3.5	3.5
Mineral mix[b]	2	2	2	2	2	2	2
Calculated analysis (g per 100 g)							
Crude protein (Nx6.25)	21.1	20.1	20.7	20.3	20.6	20.1	-
Available lysine	0.81	1.05	0.96	0.82	0.74	0.40	-
Energy content (Kcal/g)	4.17	4.17	4.14	4.18	4.12	4.16	4.16

S: Soy Protein; BY: Brewer's Yeast; BW: Brewery Waste.
a. Mineral mix provided the following, mg per Kg diet: $CaCO_3$ 10, Na_2 HPO_4 7, KCl7, $MgSO_4$ $7H_2O$ 5.9, $FeSO_4$ $7HO_2O$ 1, $MnSO_4$ H_2O 0.25, KIO_3 0.01, ZnO 0.084, $CuSO_4$ 0.01, Na SeO_3. $15H_2O$ $2.2x10^{-4}$, Co acetate H_2O 2.4 x 10^{-4}, Na_2 MoO_4. $2H_2O$ 0.008.
b. Vitamin mix provided the following per kg diet: niacin 44 mg, calcium pantothenate 13.9 mg, pyridoxine HCl 5.5 mg, riboflavin 5.29 mg, thiamin HCl 4.18 mg, folic acid 1.8 mg, menadione 1.94 mg, biotin 0.28 mg, cyanocobalamin 11 µg, choline chloride 1.54 g, vitamin E 15 IU, vitamin D_3 1000 IU, vitamin A 11000 IU.

At the end of the experiment the birds were sacrified by decapitation, blood was collected and livers and kidneys were dissected. The tissues were immediatly frozen (-20 °C). Plasma, kidney and liver uric acid were assayed by the enzymatic method (11)); the data were corrected by the nitrogen intake as recommended previously (12). Liver lipids were extracted with chlroform: methanol (1:1) according to the method of Folch et al (13). Tissue and plasma total lipids, cholesterol and triacylglycerols were determined using commercial kits (Heiga, Caracas).

The data were analyzed by one-way analysis of variance (14). The means were compared by Duncan's multiple range test (15). The probability level for statistical significance was kept at 5 %.

RESULTS

The results of proximate analysis of the protein sources are shown in Table 2. Dry brewery waste contains comparable quantities of protein, fat and crude fiber to those of numerous commercial products used in animal feeds. This could result from the progressive concentration of the remaining cereals during the process of beer production and also, by the further dehydratation of the waste. The amino acid composition indicated that, except for the sulphur-containing amino acids, which were high, the dry brewery waste has lower concentrations of essential amino acids than either soy of brewer's yeast protein.

TABLE 2
Chemical analysis of the protein sources

COMPONENT (g per 100 g)	Soy Protein	Brewer's yeast		Brewery waste
Dry matter	94.4	94.1		93.6
Crude protein (Nx6.25)	89.4	44.8		55.1
Ether extract	0.7	1.0		13.5
Ash	3.9	6.8		2.1
Crude fiber	0.6	1.5		3.6
Available lysine	3.2	1.95	Heated	0.05
			Liophylized	2.0
AMINO ACIDS (g/16 g N)				
Aspartic acid	10.0	13.2		4.8
Threonine	3.2	6.6		2.4
Serine	3.6	5.4		3.1
Glutamic acid	13.9	16.3		14.1
Proline	4.9	8.0		6.5
Glycine	2.8	4.3		2.4
Alanine	3.7	9.4		3.0
Cysteine	0.1	0.3		1.3
Valine	4.3	7.6		3.2
Methionine	0.7	0.9		1.5
Isoleucine	4.5	6.7		2.5
Leucine	6.7	8.8		5.5
Tyrosine	3.1	3.8		2.7
Phenylalanine	4.4	6.1		4.5
Histidine	2.1	2.8		1.5
Lysine	4.4	6.5		2.2
Arginine	6.4	8.0		4.3

The effect of adding dry brewery waste to he diet on the performance of broiler chickens is presented in Table 3. It shows that the inclusion of brewery waste as a complementary protein into the diets at a 20 percent level had no significant adverse effects on growth, feed consumption, feed efficiency, PER and NPR values of the chickens, nor on their concentrations of uric acid in liver, kidney and plasma. The addition of 40, 50 and 100 percent of brewery waste to the diet resulted in a progressive decrease in growth, feed consumption, PER and NPR values of the diets. The same Table shows that kidney, plasma and liver uric acid increased only in the group fed purely with brewery as a protein supplement.

TABLE 3
Performance of broiler chickens fed brewery waste from 0 to 3 weeks

	Diet Protein Mixture					
S	100	50	30	10	0	0
BY	0	50	50	50	50	0
BW	0	0	20	40	50	100
Feed intake per chick (g)	454.7±25.7[a]	467±32.6[a]	447.7±28.8[a]	446.7±28.8[a]	401.7±8.6[b]	218.6±28.7[c]
Body weight gain per chick (g0	252.6±27.2[a]	235.4±24.5[a]	218.3±31.9[a]	197.1±23.6[b]	154.4±39.3[b]	54.7±12.8[c]
Feed efficency	0.56±0.03[a]	0.50±0.03[a]	0.49±0.04[a]	0.44±0.04[b]	0.38±0.04[b]	0.25±0.04[c]
PER	265±0.22[a]	2.51±0.16[a]	2.43±0.22[a]	2.20±0.21[b]	1.89±0.19[c]	1.21±0.18[c]
NPR	2.81±0.23[a]	2.62±0.10[a]	2.58±0.19[a]	2.32±0.17[b]	2.08±0.20[b]	1.66±0.35[c]
Liver uric acid per chick (mg per 100 g x N intake)	77.8±22.2[a]	86.2±22.2[a]	61.2±±11.1[a]	66.7±25.0[a]	77.8±8.3[a]	161.9±36.9[b]
Kidney uric acid per chick (mg per 100 g x N intake)	106.4±8.3[a]	116.8±11.1[a]	111.1±11.1[a]	111.2±16.7[a]	127.9±22.2[b]	222.4±25.0[c]
Plasma uric acid per chick (mg per 100 g N intake)	4.2±0.4[a]	4.3±0.8[a]	4.2±0.5[a]	4.2±0.5[a]	4.3±0.5[a]	5.2±0.8[b]

S: Soy protein; BY: Brewer's yeast; BW: Brewery waste.
Values are means ± SD of 7 chicks per group. Means bearing different letters are significantly different ($p<0.05$) according to Duncan's multiple range test.

As indicated in Table 4, liver and plasma lipids did not change in response to dietary brewery waste with the exception of liver triacylglycerols in the groups fed with more than 40 percent brewery waste.

TABLE 4
Plasma and liver lipids of broiler chickens fed brewery waste from 0 to 3 weeks

	Diet Protein Mixture					
S	100	50	30	10	0	0
BY	0	.50	50	50	50	0
BW	0	0	20	40	50	100
Plasma total lipids (mg per 100 ml)	572±42	567±48	570±41	571±33	570±44	574±50
Plasma cholesterol (mg per 100 ml)	132±14	131±25	134±23	128±31	132±34	122±22
Plasma triacyglycerols (mg per 100 ml)	51±6	53±6	49±5	52±7	51±5	50±5
Liver total lipids (mg per g)	31.7±6.5	32.5±3.3	33.7±2.3	33.7±4.4	35.2±2.9	34.6±3.5
Liver cholesterol (mg per g)	2.2±3	2.4±6	2.3±3	2.2±3	2.3±4	2.1±2
Liver triacyglycerols (mg per g)	6.5±11	5.8±7	5.6±3	5.9±7	7.2±13*	7.7±12*

S: Soy protein; BY: Brewer's yeast; BW: Brewery waste.
Values are means ± SD of 7 chicks per group. *Significant difference ($p<0.05$).

DISCUSSION

Complementation among proteins with different composition of essential amino acids is a good approach for improving dietary protein quality. It was demonstrated previously that brewer's yeast protein can replace 50 % of the soy protein in a soy supplemented diet prepared for growing chickens (1). Therefore, in this study we examined the effect of replacing more of the soy protein with a solid brewery waste, keeping the concentration of brewer's yeast constant at 50% of the total dietary protein since this was the maximal level of replacement found in the previous trial.

As can be seen from Tables 3 and 4, in this study brewery waste could replace 20 percent of soy protein present in the diet, without any deleterious effect upon plasma and liver uric concentration. Moreover, plasma and hepatic lipids were not affected by dietary treatment. These results contrast with the hypercholesterolemic effect observed in rabbits fed 50 % brewer's yeast protein (16), indicanting that in the chicken there was not relationship between cholesterol metabolism and yeast intake.

The similar nutritive quality observed in the 20% brewery waste diet and the control diet, could be associated, party, to the fact that the available lysine concentration was the same in both diets. This available lysine concentration was in agreement with the value (≤1.01 %) reported by Han and Baker (17) as the requirement to obtain the maximum weight in growing chicken.

High brewery waste concentrations provoked nutritive quality reduction of the diet and the hepatic triacyglycerols. Heating of the brewery waste reduced 95.7 percent of the available lysine when compared with liophylized brewery waste (Table 2); accordingly a significant decrease of available lysine was observed in diets containing high levels of waste (Table 1). Anderson et al (18) have demonstrated that heating canola meal fed to chickens, decreased the availability of lysine and the digestibility of the remainder lysine. Therefore, the inability of the brewery waste to provide dietary protein over a 20 percent level, could be attributed to the decrease in bioavailability of lysine, and other essential amino acids, due to the heating. Sugahara and Kubo (19) showed that the decrease of the total energy retentions in chickens, caused by single deficiencies of lysine or sulphur-containing amino acids, were associated with the decreased feed intake. In our study, the lowest feed intake was observed in the groups fed diets containing the lowest lysine levels. However, the lysine bioavailability did not seem the sole factor involved with the dietary nutritive value decrease at brewery waste concentrations higher than 20 % since the diet with 40 % of brewery waste had a lysine concentration (0.82 %), similar to control diet (0.81 %). Thus, the total dietary essential amino acids deficiency was prevented only when the proportions of soy-yeast: brewery waste used in the protein mixture were 30:50:20 percent, respectively.

These results contrast with those reported for rats by Elias and Bressani (20) to complement Torula yeast with vegetable proteins such as corn, sesame and cotton.

They found complementation levels for yeast protein with 60 percent corn protein, 40 percent sesame protein and 80 percent cotton protein.

Doubtless, the heating of both, the brewery waste and the brewer's yeast limited the complementation among this protein sources, the brewery waste being more affected. However, the present study provides quantitative information on the appropriate combination of both protein sources. Thus, the proportions here reported would substitute 70 percent of the soy proteins in rations for poultry. A higher complementary level could be achieve using middler conditions of drying the brewery waste.

In conclusion, yeast and brewery waste could be also considered, together with other destilery derivatives (21), as economical protein sources in feed for chickens.

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