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EFFECT OF BHT (BUTYLATED HYDROXYTOLUENE) ON THE PROTEIN VALUE OF HERRING MEAL FOR THE YOUNG RAT

by

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INTRODUCTION

Autoxidation of the fat contained in herring meal may cause spontaneous heating of the meal. Addition of small amounts of antioxidants have been shown to prevent these reactions (MEADE, 1956; MEADE & MC INTYRE, 1957; AURE, 1958; BAKKEN, 1958; SPARRE, 1958). Meals with BHT (butylated hydroxytoluene) added were tested as protein sources for chicks by LAKSESVELA (see SPARRE, 1958) and by MARCH, BIELY, GOUDIE, CLAGGET & TARR (1961) who found no effect on the protein quality of the antioxidant. Some of the meals tested by LAKSESVELA (l.c.) were also tested in nitrogen balance studies with rats. In contrast to the chick experiments these experiments also included measurements of protein digestibility. The present paper summarizes these experiments with rats.

MATERIALS

Expts 1 (a—d) Seven out of a total of 24 meals produced on the same day from small summer herring were tested in nitrogen balance experiments at 1, 5, 6 and 23 weeks after the production date. Three meals were tested at each time and compared with spray dried egg albumin. The antioxidant was added either to the concentrated stickwater in amounts corresponding to 0.03 and 0.1% in the meal or after the drying process at the 0.03% level. In Expt 1a three unpelleted meals were compared, a control without added BHT (no. 3), one with 0.03% (no. 11) and one with 0.1% (no. 19). The antioxidant was added through the concentrated stickwater. In Expt 1b the control meal without added BHT (no. 3) was compared with the same meal in pelleted form (no. 4) and with a meal which had maximal addition of BHT, 0.1% through the stickwater and 0.03% after the drying process (no. 24). In Expt 1c the control meal (no. 3) was compared with a meal to which had been added 0.03% BHT after the drying process (no. 8) and a meal to which had been added

Table 1. *Description of the herring meals used in Expts 1 (a—d).*

Meal no.	% BHT in meal		Age of meal when tested (weeks)				Protein (%)
	Added to stickwater	Added to meal	a	b	c	d	
3	—	—	1	5	6	23	70.4
4*	—	—		5			69.9
8	—	0.03			6		67.6
11	0.03	—	1		6		67.0
19	0.1	—	1				68.9
20*	0.1	—				23	65.5
24	0.1	0.03		5		23	68.7

* Ground pellets.

0.03% BHT through the stickwater (no. 11). In Expt 1d the control meal (no. 3) was compared with meal no. 24 and a pelleted meal which had 0.1% BHT added through the stickwater (no. 20). The treatments compared are summarized in Table 1.

Expt 2 (a—c) Four of the meals produced from winter herring described by AURE (1958) were tested in nitrogen balance experiments when they were newly produced and 9 and 18 weeks after the production date. Meal nos. 7A and 9A were produced in a flame drier and meal nos. 7B and 9B in a steam tube drier. BHT was added at the 0.03% level to the press cake and 0.03% after the drying process to meals 9A and 9B (Table 2).

Expts 3 I (a—d) Two meals produced in a flame drier were compared with two pelleted meals produced in a Dyno drier. To one meal of each type 0.03% BHT was added after the drying process. The meals were tested

Table 2. *Description of the herring meals used in Expts 2 (a—c).*

Meal no.	% BHT in meal		Production method*	Age of meal when tested (weeks)			Protein content %
	Added to press cake	Added to meal		a	b	c	
7A	—	—	Flame	0	9	18	74.8
7B	—	—	Steam tube	0	9	18	72.9
9A	0.03	0.03	Flame	0	9	18	75.6
9B	0.03	0.03	Steam tube	0	9	18	73.2

* Flame drier method H and Steam tube drier method I as described by NJAA, UTNE & BRÆKKAN (1966).

Table 3. *Description of the herring meals used in Expts 3I (a—d) and Expts 3II (a—c).*

Meal*	BHT added to the meal %	Production method**	Age of meal when tested (weeks)				Protein content %
			a	b	c	d	
DOP	—	Dyno	1	11	19	5	74.1
DAP	0.03	—	1	11	19		74.5
FOM	—	Flame	1	11	19		73.2
FAM	0.03	—	1	11	19		74.5
DOM	—	Dyno	2	10	18		74.2
DAM	0.03	—	2	10	18		71.6
FOP	—	Flame	2	10	18		73.7
FAP	0.03	—	2	10	18		74.5

* The coding of the meals are as follows: D = Dyno drier; F = Flame drier; O = Without antioxidant; A = With antioxidant added; P = Ground pellets; M = Meal

** Dyno drier method L and Flame drier method K as described by NJAA, UTNE & BRÆKKAN (1966).

when they were 1, 11 and 19 weeks old. The pelleted meal without added BHT heated spontaneously in the storing bins. After two days the temperature was 80°. The pellets were then spread on the floor for cooling and one laboratory sample of the meal which did not heat was compared in a separate experiment with the heated sample five weeks after the production date (Expt 3 I d).

Expts 3 II (a—c) These experiments were similar to Expts 3 I (a—c). The meals produced by the flame drier were pelleted and those produced by the Dyno drier were not. These meals were tested when they were 2, 10 and 18 weeks old. The meals used in Expts 3 I and 3 II are described in Table 3. They were all produced from winter herring.

METHODS

The nitrogen balance experiments were performed with 5-day collection periods following a 4-day preliminary period. Four groups of rats were used so that four protein sources were compared each time. New rats were used in each experiment. The food intake was kept at 10 g/rat/day except in Expt 1b where it was 8 g/day. The protein content of the diet was about 8%. The rats were kept at 22±1°C. The details of the methods are described by NJAA (1959, 1963) and by NJAA, UTNE & BRÆKKAN (1966).

RESULTS

The results are given in Table 4.

In Expt 1a when meal no. 3 without added BHT was compared with meals nos. 11 and 19 which had two different levels of BHT, 0.03 and 0.1% added through the stickwater, no significant differences were observed. Thus, the presence of BHT during the production period seemed to be of no advantage. In Expt 1b the nitrogen balance was significantly lower for the pelleted meal no. 4 than for the mean of the two unpelleted meals no. 3 and no. 24 ($p<0.05$) whereas the two unpelleted meals were not significantly different. Meal no. 24 with maximum amounts of BHT tended, however, to show a better nitrogen balance than the meal without added BHT, whereas the digestibilities were not significantly different. In Expt 1c meal no. 8 with 0.03% BHT added after the drying process showed a significantly better digestibility than

Table 4. *Apparent digestibility* (D_a), *percentage nitrogen balance* (*Bal%*) *and calculated*

Expt no.	Meal no.	N-intake mg per day				D_a				Bal %			
		a	b	c	d	a	b	c	d	a	b	c	d
	3	126.2	101.6	132.2	132.4	77.7	76.2	76.5	75.0	37.7	38.9	40.7	37.4
	4		100.4				76.2				35.4		
	8			129.0				78.9				43.5	
1	11	126.3		117.3		77.7		76.5		37.5		44.0	
	19	125.6				76.3				38.0			
	20				129.8				76.5				34.8
	24		100.8		131.6		77.7		78.1		41.7		45.1
	E—a	125.9	101.4	126.2	127.5	83.3	82.9	82.8	82.5	63.4	57.0	66.8	64.3
	7A	125.8	127.6	127.7		77.4	78.7	76.9		42.6	50.2	43.2	
2	7B	124.0	128.5	127.1		80.1	76.7	76.8		41.7	49.4	43.5	
	9A	126.6	129.5	128.3		78.8	77.3	78.5		38.1	47.9	43.3	
	9B	125.2	126.6	128.7		81.6	79.4	78.8		42.4	50.1	44.9	
	DOP*				123.7*				74.4*				39.0*
	DOP	126.1	128.0	124.7	124.6	80.3	79.2	73.9	77.2	42.9	48.2	38.0	42.7
3I	DAP	126.4	129.5	125.5		80.1	80.9	77.3		42.7	51.1	39.4	
	FOM	127.5	128.9	126.1		81.5	80.9	76.4		39.8	48.8	37.7	
	FAM	125.8	127.4	120.8		82.0	82.1	75.8		43.2	51.2	33.9	
	DOM	128.7	134.8	125.1		79.3	79.5	74.7		38.9	41.1	38.5	
3II	DAM	128.2	130.2	126.5		78.2	77.1	77.3		45.2	41.4	42.2	
	FOP	128.2	127.8	125.8		80.2	79.6	78.0		41.4	40.4	42.7	
	FAP	128.1	131.1	125.3		79.7	79.5	78.3		41.0	42.3	40.1	

* Spontaneously heated DOP compared with an unheated sample when the meals were

the mean for meal nos. 3 and 11 which had either no BHT or 0.03% BHT added through the stickwater ($p<0.01$). There were no significant differences for the nitrogen balance values although the meals with added BHT tended to be slightly better than meal no. 3. This tendency was also reflected in the weight gains. In Expt 1d meal no. 24 with the highest addition of BHT showed a significantly better digestibility than the mean of the two other meals (nos. 3 and 20) of which one had no BHT added (no. 3) and one (no. 20) had 0.1% added through the stick-water ($p<0.05$). Meal nos. 3 and 20 showed no significant difference in digestibility values. The nitrogen balance value of meal no. 20 was significantly lower than the mean for meal nos. 3 and 24 ($p<0.001$), and of these no. 24 showed a significantly better nitrogen balance value than no. 3 ($p<0.001$). The differences observed between meal no. 3 without added BHT and other meals were only partly due to a detori-

net protein value (NPU_c) *of herring meal protein with and without addition of BHT.*

NPU_c				Body weight g				Weight gain (g per 5 days)			
a	b	c	d	a	b	c	d	a	b	c	d
60.3	63.2	62.0	59.2	74.6	61.0	73.5	75.5	5.2	4.0	4.0	5.7
	60.6				63.7				4.0		
		65.4				73.8				5.0	
59.9		67.8		73.8		72.2		5.8		4.7	
60.8				75.0				4.3			
			57.1				76.0				6.5
	66.6		67.6		62.2		78.2		6.0		7.0
87.4	82.8	90.6	88.5	80.0	66.0	79.6	82.4	7.3	6.0	9.2	8.5
64.5	72.8	67.3		71.2	75.3	82.3		6.0	8.2	0.8	
64.0	71.6	67.4		71.1	74.3	81.3		5.5	7.6	0.3	
59.6	69.9	66.8		69.5	74.4	80.1		4.7	7.6	1.2	
66.5	72.9	68.2		71.4	75.3	79.7		5.5	7.4	1.0	
			62.9*				78.0*				6.0*
67.0	70.8	62.7	66.4	81.3	75.5	82.6	78.4	6.2	9.4	5.2	6.8
66.6	73.3	64.1		80.6	74.9	83.1		6.2	9.8	6.5	
63.5	71.1	61.8		80.4	74.8	81.5		6.0	9.2	6.3	
67.4	73.7	59.1		81.2	74.8	81.4		7.0	10.4	6.5	
61.8	63.5	62.8		77.5	80.6	81.0		8.0	7.0	8.0	
68.2	64.6	66.4		78.0	80.8	82.1		6.7	6.8	5.2	
64.5	63.9	66.4		78.7	80.0	79.0		7.7	7.0	8.7	
64.1	65.5	64.8		78.4	80.9	83.5		6.8	7.0	9.3	

five weeks old.

ation of this meal with time insofar as the digestibility value tended to decline. The nitrogen balance values were remarkably constant for this meal over the 23 weeks of the experiments.

In Expt 2a there were significantly higher digestibilities for the meals containing BHT ($p<0.05$) and for the meals dried in a steam tube drier ($p<0.001$) when the meals were newly produced. The differences between nitrogen balance values were not significant; when the meals were 9 and 18 weeks old no significant differences were observed (Expts 2b and c).

In Expts 3 I significant differences were only observed between digestibilities. After 1 and 11 weeks the meals produced by flame drier were significantly better digested than those produced by Dyno drier and pelleted ($p<0.01$). No significant difference was observed when the meals were 19 weeks old. The effect of added BHT was only significant when the meals were 11 weeks old ($p<0.01$). The difference involved were, however, small. For all meals the digestibilities tended to decrease as they became older.

The comparison between a spontaneously heated sample of Dyno dried pelleted meal without added BHT with an unheated sample of the same meal (Expt 3 I d) showed a significant reduction in the protein digestibility of the heated sample of about 3 percentage units ($p<0.05$). The corresponding difference between the nitrogen balance values was not significant.

In Expts 3 II no significant differences were observed between digestibilities or nitrogen balances.

DISCUSSION

Few studies report on the effect of BHT or other antioxidants on the protein quality of fish meals. In most instances the studies have been concerned with the effect on the lipid fraction of the meals (Lea, Parr & Carpenter, 1958; March, Biely, Clagget & Tarr, 1962; Flanzy, Roquelin, Pihet, Meurot & Boufflers, 1962).

The results obtained in the present experiments with young rats generally confirm the conclusion of Laksesvela (see Sparre, 1958) and of March *et al.* (1961) based on experiments with chicks. They found that the amounts of BHT used had little effect on the protein quality of herring meal. In the present investigation, however, small effects of BHT were observed in a few instances sufficiently consistent as to warrant comments. In Expt 1 addition of 0.03% BHT to the meal after the drying process significantly improved the protein digestibility as compared with the control meal, both when it was the only addition (meal no. 8, Expt 1c) or when it was added to a meal with 0.1% BHT added before the drying process (meal no. 24, Expt 1d), the latter addition alone had no effect (meal no. 20, Expt 1d). Meal no. 24 was also slightly better digessted than the control meal in Expt 1b, but the effect was not significant. In Expts 1a and c it was confirmed that addition of BHT before the drying process was without any effect. The nitrogen balance value of meal no. 24 was significantly better than that of the control meal in Expt 1d and the value for meal no. 20 with 0.1% BHT added before the drying process was actually significantly lower than that of the control meal. The result in Expt 1b was similar for meal no. 24 but the effect was not significant. The NPU values indicated the same trend as the nitrogen balance values. Relative to the spray dried egg albumin meal no. 24 was better utilized in Expt 1b than in Expt 1d.

This improvement in protein quality when BHT is added to the meal after the drying process runs parallell to the reduction in oxygen uptake during the first 200 hours after the production of the meals (ASTRUP, AREFJORD & GLOPPESTAD, see SPARRE, 1958). Thus the improvement in protein quality may be due to a reduced rate of oxidation of the oil contained in the meals.

Pelleting of the meal after the production process seemed to have no effect on the protein utilization although it is not improbable that the low nitrogen balance value of meal no. 20 in Expt 1d was an effect of the pelleting procedure.

As a consequence of the results obtained in Expt 1 BHT was added after the drying process to all the meals tested in Expts 2 and 3.

The effect of BHT observed in these experiments were obviously of no practical concequence. It deserves mentioning, however, that BHT in a few instances improved the protein digestibility. This may be explained by the finding of MARCH *et al.* (1961) that herring meals treated with BHT contained less pepsin undigestible nitrogen than untreated meals.

The effect of different production methods were also small and of no practical consequence. In Expt 3 the possible effects of production methods are masked by the possible effects of the pelleting procedure. It is obvious in retrospect that the comparisons made ought to have been otherwise planned. The reason for the choise made was that it was presumed that both drying in a Dyno drier and pelleting would improve the protein quality as compared with flame drying and keeping the meal unpelleted. Thus it was assumed that the differences observed in Expt 3 I would be greater than in Expt 3 II. As this was not the case this problem was dropped and the experiment is reported as a preliminary test which was not followed up. In fact the indications were that contrary to expectation pelleting might accelerate the oxidation process instead of retarding it (Expt 3 I d).

The general lack of effect of BHT on the protein quality in Expts 2 and 3 was not in accord with the observed effect of BHT added after the drying process to the meals in Expt 1. This difference may be due to the different raw materials used. The small summer herring processed in Expt 1 is known to be a difficult raw material for herring meal production, the summer herring usually have a full stomach whereas the winter herring is empty. The iodine number was distinctly higher for the meals used in Expt 1 than for those used in Expt 2 (Astrup *et al.*, see Sparre, 1958; Aure, 1958). In the press cake fat the iodine number was 169 in Expt 1 and 135 in Expt 2.

The use of BHT in the Norwegian herring meal industry never passed the experimental stage. The main reason for this was the findings that BHT containing meals adversely affected the quality of slaughter chickens, egg and bacon fat (Aure, 1958; Laksesvela, see Sparre, 1958; Hvidsten, Astrup & Aure, 1965).

The problem of spontaneous heating of herring meal has not been solved, but the industry has learnt to live with it insofar as the storing conditions are so chosen as to keep the problem under control.

SUMMARY

The effect of BHT (butylated hydroxytoluene) on the protein quality of herring meal was studied in nitrogen balance experiments with young rats.

BHT at the 0.03% level in the meal improved the protein quality of meals produced from small summer herring when it was added after the drying process but was without any effect when it was added before the drying process.

BHT had only little effect on the protein quality of meals produced from winter herring.

Different production methods of the meals had little effect on the protein quality.

Pelleting of the meals seemed to be no advantage, there were indications that the protein quality might be adversely affected by this process.

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