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PIGMENTATION OF SALMONID - A COMPARISON OF ASTAXANTHIN AND CANTAXANTHIN AS PIGMENT SOURCES FOR RAINBOW TROUT

by

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ABSTRACT

Rainbow trout were fed a diet supplemented with astaxanthin and cantaxanthin. The ratios of astaxanthin to cantaxanthin were determined in plasma, faeces, and flesh. During 65 days of feeding the flesh reached a level of 4.5 mg carotenoids/kg. A linear relationship between growth and carotenoid level was found for growths below 150g/65 days. Astaxanthin seemed to be absorbed more readily from the diet and also deposited at a higher ratio than cantaxanthin in the flesh. Pylorus seems to be the site for absorption of astaxanthin.

INTRODUCTION

The pink to red pigment in the flesh of wild salmonids is of dietary origin and due to accumulation of the carotenoid astaxanthin. The demand for pigmented flesh in farmed rainbow trout (Salmo gairdneri) and Atlantic salmon (Salmo salar) has focussed investigations on products containing astaxanthin of mainly crustacean but also microbial sources (Torrissen and Nævdal, 1984). Another carotenoid, cantaxanthin, have nevertheless become the predominant pigment source for cultured salmonids. This is because cantaxanthin is absorbed and deposited well, gives nearly the same coloration of the flesh as astaxanthin but also because this pigment has been synthesized in commercial scale for more than one decade and is available as a gelatin stabilized micro-dispersed granulate containing 10 % cantaxanthin (Carpohyll red, Hoffman La Roche, Basle, Switzerland). This granulate make it possible to incorporate cantaxanthin in any diet at controllable levels without significant influence on the dietary quality.

Hata and Hata (1972) proposed that introduction of hydroxyl groups in the carotene skeleton results in better absorption and accumulation of the carotenoid. If so, astaxanthin, the

dihydroxycantaxanthin (Fig. 1), should be absorbed and accumulated better than cantaxanthin. However, Torrissen (1978) and Tidemann el al (1984) reported that synthetic cantaxanthin was accumulated at a higher rate rate than astaxanthin from shrimp (Pandalus borealis) waste.

Recently synthetic astaxanthin has also been commercially available. Increasing interest has focused on this natural salmonid pigment proposed to take over cantaxanthins role as the dominant salmonid pigment. This synthetic, unesterified, astaxanthin has been reported by Tidemann et al (1984) and Foss et al (1984) to have superior accumulation rates in the flesh of rainbow trout than synthetic cantaxanthin.

The aim of the present experiment was to compare absorption and deposition of astaxanthin and cantaxanthin in order to confirm the previous observations conserning accumulation rate of synthetical astaxanthin compared to cantaxanthin, and to investigate at what metabolic level the rainbow trout discriminate astaxanthin and cantaxanthin, digestibility or accumulation in the flesh.

MATERIAL AND METHODS.

About 50 unpigmented rainbow trout (Salmo gairdneri) were individually labeled by Floy Anchor Tags FD-67C (Floy Tag & Manufactoring, Inc., Seattle, USA) and transferred to a 3 m^3 circular fibre glas tank supplied with sea water. The temperature of the sea water ranged from 9 to 12 °C. During a two week acclimatization period, the fish were fed a commercial dry pelleted feed free from carotenoids in significant amounts, and thereafter fed the experimental diets to satiation three times per day for a total of 65 days.

The composition of the experimental diet are shown in table I. The synthetic astaxanthin (5%) and cantaxanthin (10 %) granulate were supplied by Hoffman La Roche, Basle, Switzerland. Both granulates were dissolved in warm water (about 60 C) before addition (1:100) to the feed which was finally extruded through a meat grinder with 5 mm holes and stored frozen. On Days 17, 38 and 65 the fish were anaesthetized with benzocain and the individual weight recorded.

Faeces samples were collected by stripping (Austreng, 1978) and blood collected from the postcardinal vein by evacuated tubes containing 0.1 ml EDTA/ 5 ml.

Ten fish were sampled for carotenoid analysis. These were gutted and skinned and the flesh frozen for later carotenoid examination. The blood was centrifuged at 2000/rpm for 10 min, the serum fraction collected and the plasma frozen.

At the end of the experiment, the gastrointestinal tract of four fish were divided in five sections; stomach, pylorus, anterior midgut, posterior midgut and hindgut (faeces), and the content of each section collected for carotenoid analysis.

The samples for carotenoid analysis were homogenized and 0.75 to 1.5g collected. The samples were repeatedly extracted by acetone. The acetone and water were evaporated by vacuum and the carotenoid extract diluted volumetrically with hexane.

The carotenoids were separated and quantified by HPLC using a Supelco 25 cm, 4.6 mm, LC-CN column. Hexane (76%), acetone (7%) and isopropylacetate (17%) were used as eluants (Foss et al, 1984) and absorption determined at 476 nm. For quantification of both astaxanthin and cantaxanthin, cantaxanthin was used as the standard. The relative amount of astaxanthin and cantaxanthin was calculated by the area of each absorption peak.

The dry matter was determined by freece drying, protein (N X 0.25) by the Kjeldahl method, fat by dicthylether ectraction in soxhlet apparatus, ash by combusion at 550°C and carbohydrates by difference.
The data description and statistical analysis was carried out by using the RS/1 program (Bolt Beranek and Newman, Inc, Cambridge, USA).

RESULTS.

The proximate analysis of the diet is shown in Table 1.

The deposition of astaxanthin and cantaxanthin in the flesh is shown in Fig. 2. A total of 4.5 mg/kg of cantaxanthin and astaxanthin were deposited in the flesh during the 65 days of feeding, of which was 2.6 mg/kg cantaxanthin and 1.9 mg/kg was astaxanthin. The amounts of both increased during the whole experiment, but the rate of accumulation decreased between Day 38 and Day 65. The level of cantaxanthin was significantly higher than astaxanthin in all periods ($P<0.05$, paired t-test).

The level of carotenoid accumulation as a function of growth rate is shown in Fig. 3. There seemed to be a linear relationship between growth rate and carotenoid accumulation in the flesh up to a growth of 150 g during the 65 days of feeding. For cantaxanthin the slope was 0.012 and $r^2 = 0.9999$, while for astaxanthin the slope was 0.0119 and $r^2 = 0.992$. However at higher growth rates the carotenoid accumulation seemed to reach a plateau.

The ratio of cantaxanthin to astaxanthin in feed was 1.7, significantly lower than plasma and faeces ($P<0.05$, t-test unequal variances) but significently higher than in flesh ($P<0.05$ by Mann-Whitney test, Fig. 4).

Figure 5 shows the cantaxanthin to astaxanthin ratio in five segments of the gastrointestinal tract, stormach, pylorus, anterior midgut, posterior midgut and hindgut. The ratio increased from 1.8 in the stomach to 2.7 in the pylorus and then decreased to 2.5 in anterior midgut and 2.25 posterior midgut to finally 2.0 in the hindgut (faeces).

DISCUSSION

The ratio of cantaxanthin to astaxanthin in the diet of 1.7 is a result of an astaxanthin concentration below the manufacturers decleared level of 5% astaxanthin in the carophyll pink. The level of cantaxanthin in the diet was found to 48 mg/kg while the astaxanthin concentration was 27 mg/kg (Table 1).

The level of cantaxanthin in the flesh was significantly higher than astaxanthin, due to the different levels of cantaxanthin and astaxanthin in the diet. However, the ratio in the diet was significantly higher than the cantaxanthin to astaxanthin ratio in the flesh (1.4), showing that astaxanthin is deposited in the flesh more readily than cantaxanthin. This confirms the hypothesis of Hata and Hata (1972) that introduction of hydroxyl groups in the carotenoid skeleton improves accumulation and supports the observations by Tidemann et al (1984) and Foss et al (1984).

However, the result is contrary to those of Torrissen (1978) who found superior accumulation of cantaxanthin (Carophyll red, Hoffman La Roche, Basel, Switzerland) over astaxanthin from Calanus finmarchicus in the flesh of rainbow trout when the Carophyll red granulates were dissolved in warm water before addition to the feed. A opposite effect was found when the granulates were added directly to the wet feed. Tidemann et al (1984) also got a higher deposition with water dissolved cantaxanthin than astaxanthin from shrimp waste. This difference in observations might be explained by the esterified form of astaxanthin in C. finmarchicus and shrimp waste. In both sources 60 to 80 % of the astaxanthin usualy is esterified, which is absorbed and accumulated far less by rainbow trout than the unesterified form (Torrissen and Brækkan, 1979). It is also possible that the astaxanthin in crustaceans is less available because it is protected by the calcium structure of the shells (Torrissen et al, 1982).

As the ratio of cantaxanthin to astaxanthin in the faeces was significantly higher than the ratio in the diet. This shows that the absorption of astaxanthin in the digestive tract is higher than cantaxanthin.

The relatively high cantaxanthin to astaxanthin ratio in plasma shows an organ specificity in carotenoid preference. Considering only the digestibility of cantaxanthin and astaxanthin a lower cantaxanthin to astaxanthin ratio than in the feed should be expected because of the better absorption of astaxanthin. This significant higher ratio of cantaxanthin to astaxanthin than in the diet is probably an effect of a more efficient deposition of astaxanthin in the flesh than cantaxanthin.

The accumulation of carotenoid was linear up to a growth of 150 g (Fig. 4), but for higher growth rates this relationship decreased. If one assumes that the growth rate is proportional to food consumption, the ingested amount of carotenoids should also be proportional with the growth rate. Thus a linear relationship between growth rate and carotenoid deposition should be expected. The linear relationship up to a growth rate of 150 g regardless of time of feeding indicate that the flesh have reached a degree of saturation. The results presented by Torrissen and Torrissen (1984) and Torrissen et al (1984) indicate a saturation level of about 4 to 5 mg carotenoids pr kg flesh. This saturation level might however be influenced by genetical factors (Torrissen and Nævdal, 1984), by dietary level (Torrissen, 1985), fish size and species. In relation to species it is interesting to note that Torrissen et al (1984) shoved a level for family groups of Atlantic salmon of 4 to 5 mg/kg after 26 months feeding while Torrissen and Nævdal (1984) for families of rainbow trout reached a level of 5.5 to 6.5 after about 5 to 6 months feeding with diet supplemented cantaxanthin. This indicating that rainbow trout accumulate carotenoids better than Atlantic salmon.

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TABLE 1. Composition of the experimental diet.

Ingredients	kg/100 kg
Moist pellet meal(a)	51.9
Water	37.5
Soybean oil	3.0
Cod liver oil	0.5
Herring meal (NorSea Mink)	7.0
Carophyll red (aa)	0.050
Carophyll pink (aaa)	0.100

(a) Tess pelletmel, T. Skretting A/S, Stavanger, Norway
(aa) Carophyll red, Hoffman La Roche, Basle, 10% Cantaxanthin
(aaa) Carophyll pink, Hoffman la Roche, Basle, 5% Astaxanthin

The proximate analysis of the diet showed the following results;

cantaxanthin: 48 mg/kg, astaxanthin: 27 mg/kg, moisture: 45%, protein: 34.1%, fat: 5.6%, ash: 6.2 and carbohydrates: 9.1%.

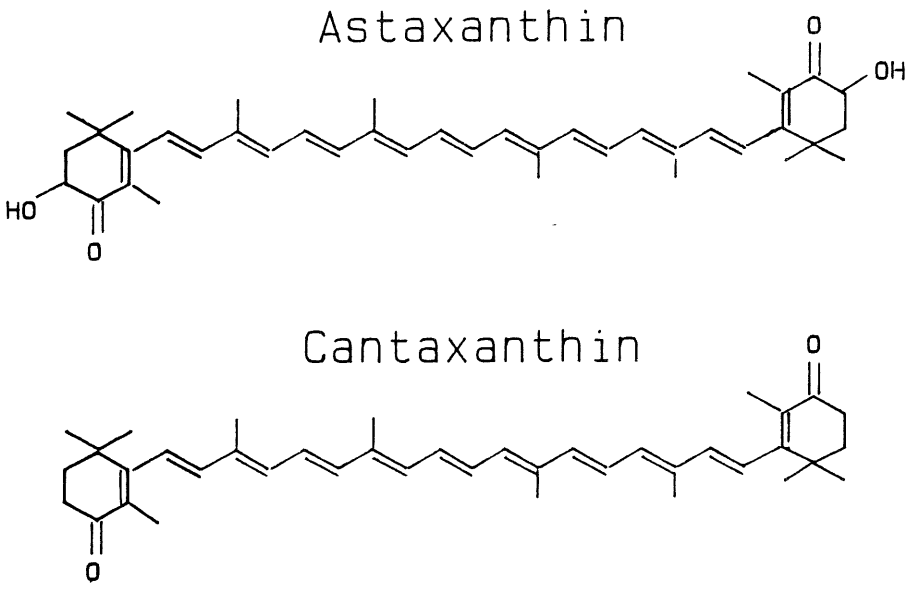

Figure 1. Astaxanthin and cantaxanthin.

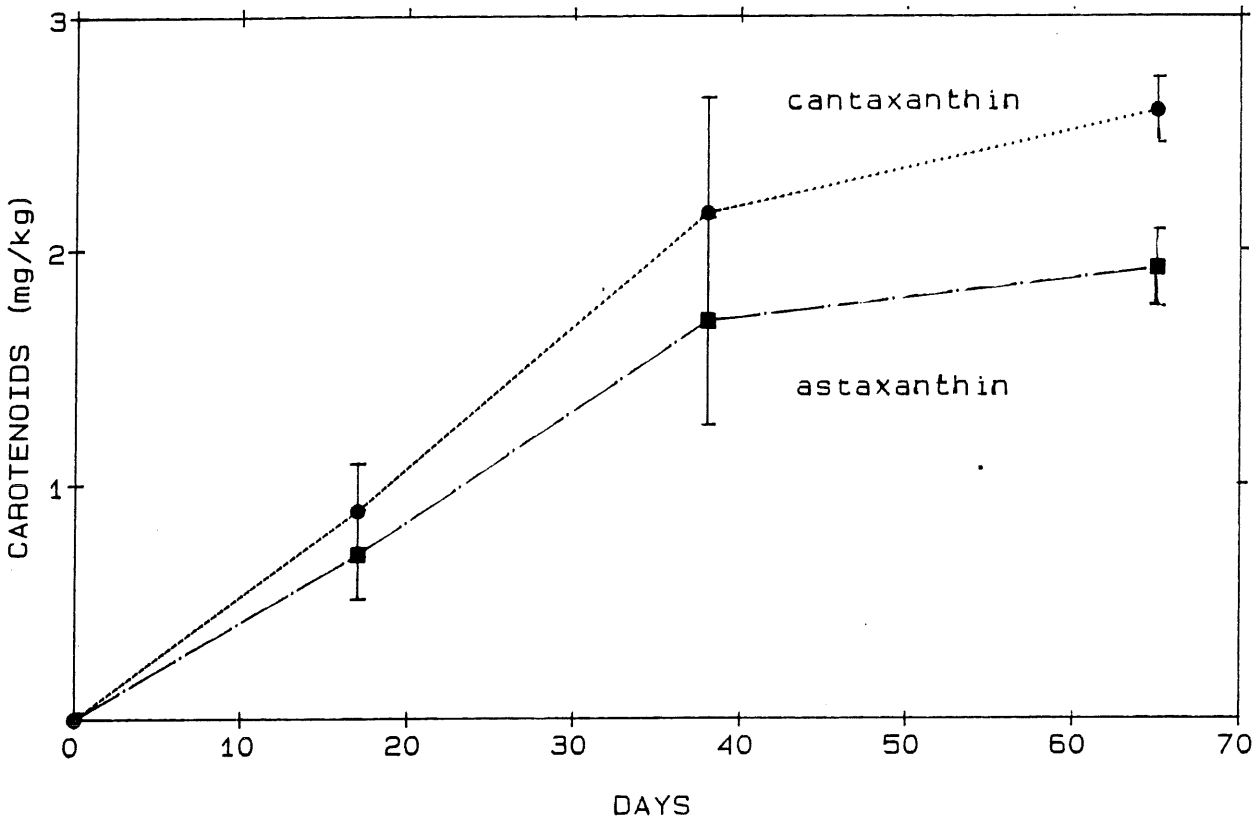

Figure 2. The deposition of cantaxanthin and astaxanthin in the flesh of rainbow trout by feeding a diet containing 48 mg/kg cantaxanthin and 27 mg/kg astaxanthin.

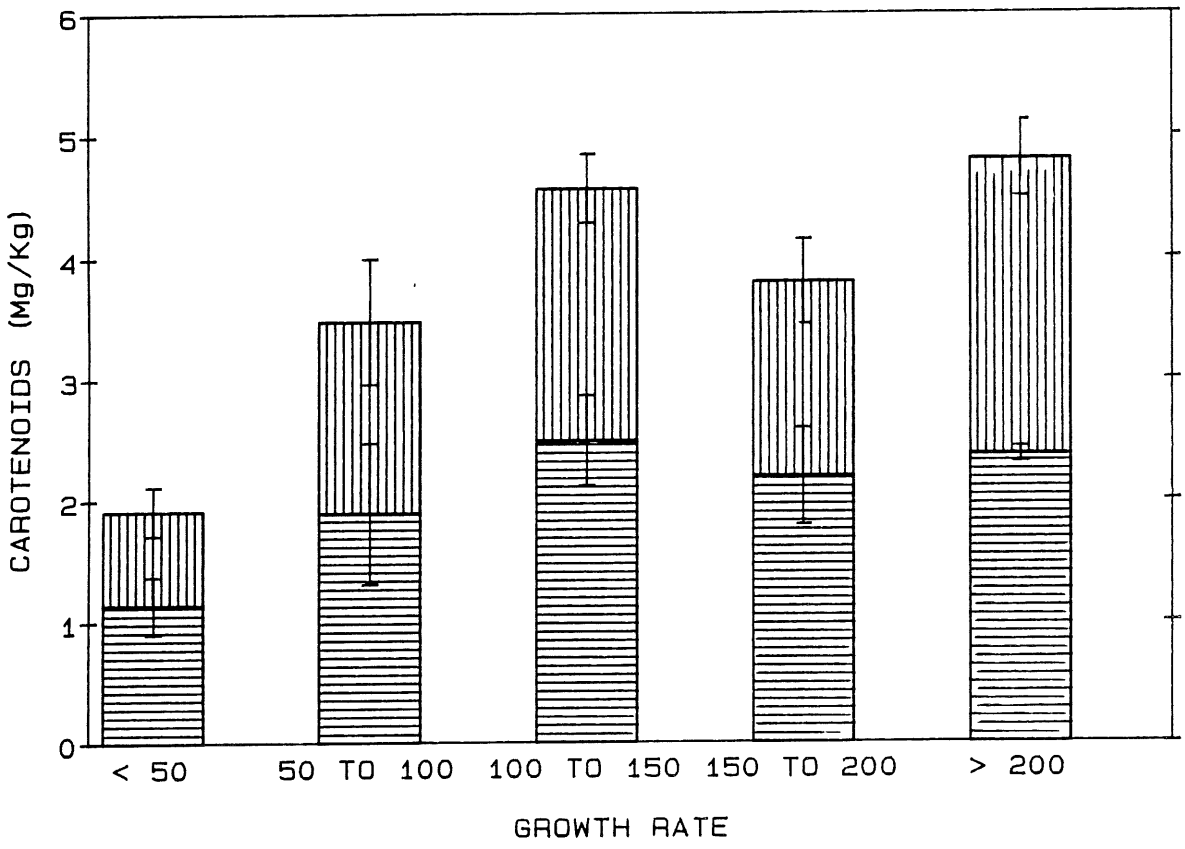

Figure 3. The level of cantaxanthin and astaxanthin in the flesh of rainbow trout as a function of growth rate (g). The horizontal bar shading shows the cantaxanthin while the vertical bar shading the astaxanthin level.

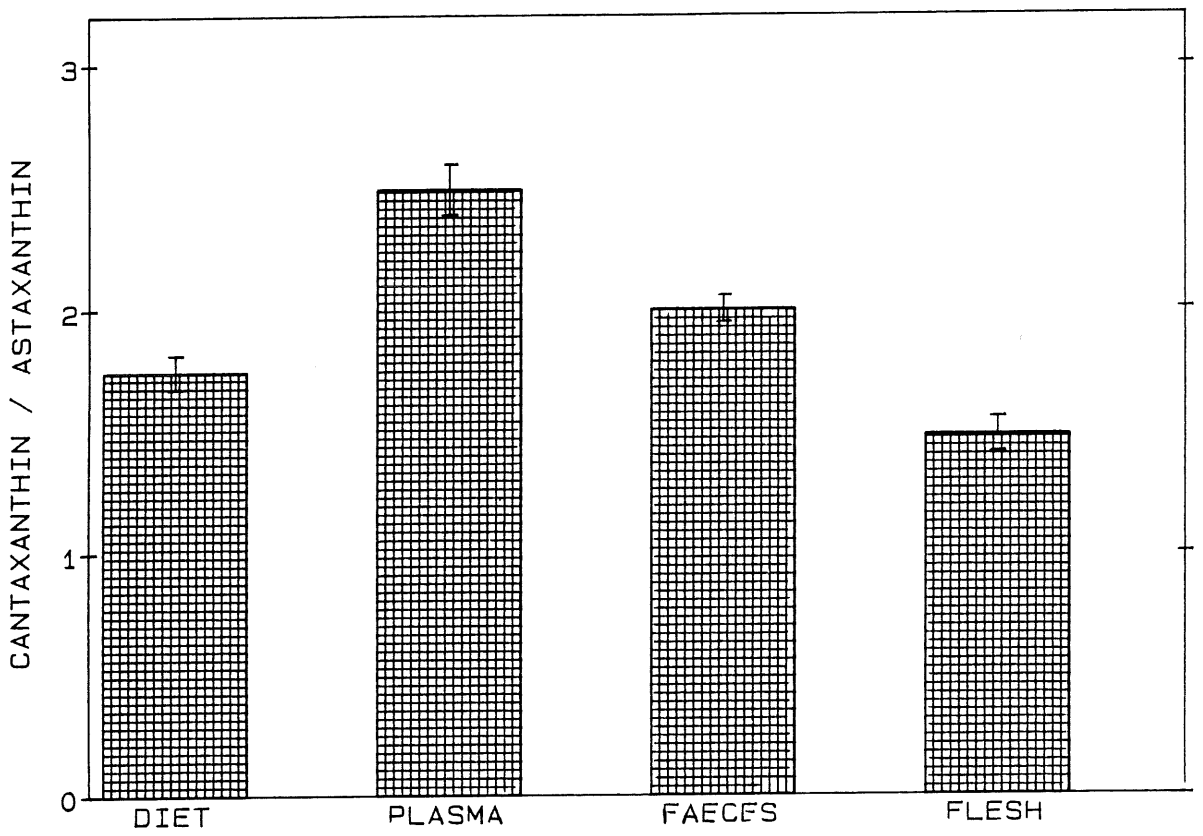

Figure 4. The ratio of cantaxanthin to astaxanthin in the feed, plasma, faeces and flesh of rainbow trout.

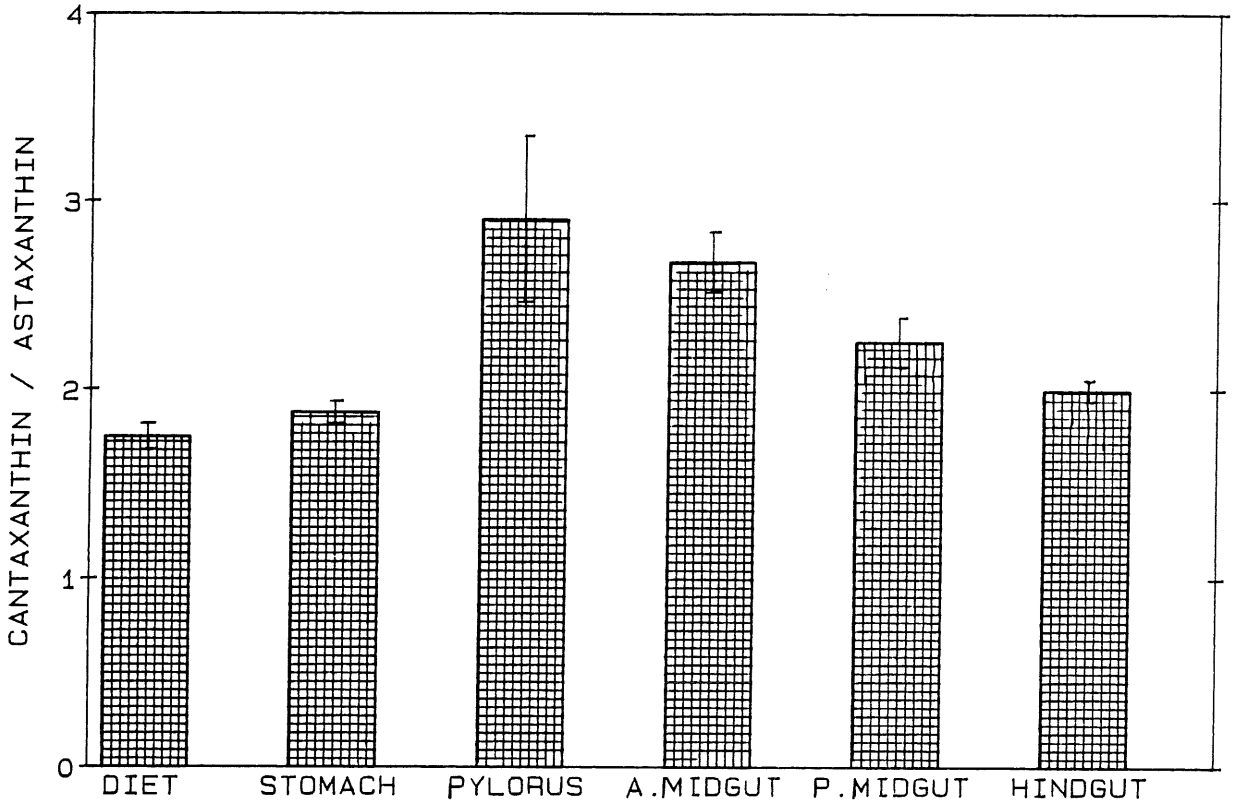

Figure 5. The ratio of cantaxanthin to astaxanthin in the content of different segments of the gastrointestinal tract.