

Effect of Protein Level and Duration of Test on Carcass Composition, Net Protein Utilization (NPU) and on Protein Efficiency Ratio (PER)¹

**Wirkung des Eiweißgehaltes der Kost und der Versuchsdauer
auf Körperzusammensetzung, Eiweißnutzwert und Eiweißwirkungsgrad**

**Effet du taux protéique et de la durée de l'essai sur la composition de la carcasse,
l'utilisation protéique nette et le coefficient d'efficacité protéique**

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In recent years, new or modified methods for the evaluation of protein quality have been developed. One of these described by *Miller and Bender* [1] is called Net Protein Utilization (NPU) and is equal to the ratio of the amount of nitrogen deposited in the tissues of rats and the nitrogen consumed. Corrections for the endogenous nitrogen are made by subtracting from the body nitrogen of the animals fed the protein under test, the body nitrogen of the animals fed a nitrogen-free diet. The experimental period for the test is 10 days and the level of protein in the diet is 10%. On the other hand, the most common biological method for protein evaluation, denominated protein efficiency ratio (PER), is carried out for 28 days using 10% protein diets. This figure represents the ratio of body weight gain over the amount of protein consumed.

The two methods are really measuring the same variable, although the procedures used are different. The factors which affect one should also affect the other since both methods are based on the ability of the

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protein to supply the animal with the quantity and quality of amino acids needed for growth and other metabolic functions.

The effect of protein level and of time on PER has been reported by *Middleton et al.* [2]. As the protein level increases, there is an increase in PER to a maximum value, which for good quality protein is about 10% of the diet [3]. Higher levels will result in lower values [4]. The protein efficiency ratio has been reported to decrease with respect to time [5], the decrease being greater for better quality proteins. Similar studies on the effect of protein level on NPU are limited [6] and there are none on the effect of time on NPU. The present study was carried out for the purpose of determining the effect of those two factors, protein level of the diet and time on NPU and PER, on casein, and several vegetable proteins. At the same time, in some of the experiments, the carcass composition of the experimental animals was studied under the different protein levels fed and at weekly intervals throughout the experimental period.

Materials and Methods

Effect of time on PER. In these studies, eight female and eight male rats of the Wistar strain from the INCAP colony were fed 10% protein diets for a period of eight weeks. The diets consisted of: mineral mixture [7], 4.0%; cottonseed oil, 5.0%; cod liver oil, 1.0%, and dextrose, 32.0%. The difference was made up with an amount of test material which provided 10% of protein and cornstarch to add up to 100 g. The diets were supplemented with 5 ml of a complete vitamin solution [8] per 100 g of diet. The animals were placed in individual all-wire cages with raised screen bottoms and fed food and water *ad libitum*. Weight gain and food intake were measured every seven days.

Effect of protein level in the diet on PER. For each protein studied, 30 male rats of the Wistar strain were distributed by weight among 5 different protein level groups. The levels of protein tested were 5, 10, 15, 20, and 30% of the diet. The diets used were of the same composition as the one described above, except that the test material was added to give different levels of protein at the expense of cornstarch. The rats were treated as before and the experimental period lasted 28 days.

Effect of time and protein level on NPU. In these studies, the effect of the two variables was studied simultaneously using 102 weanling rats of the same sex. Four protein levels were tested for four weeks. Twenty-four rats were assigned to each protein level and at the end of 7, 14, 21, and 28 days, a group of 6 rats was sacrificed. Six rats were sacrificed at the beginning of the study. The diets used were equal to those already described and the experiment was carried out as before. The individual animals being sacrificed were analyzed for moisture, fat, protein and ash. Net protein utilization was calculated at the end of each week, using the composition of the initial group as a correction factor for the protein deposited and the protein intake during the period the animals were consuming the diet.

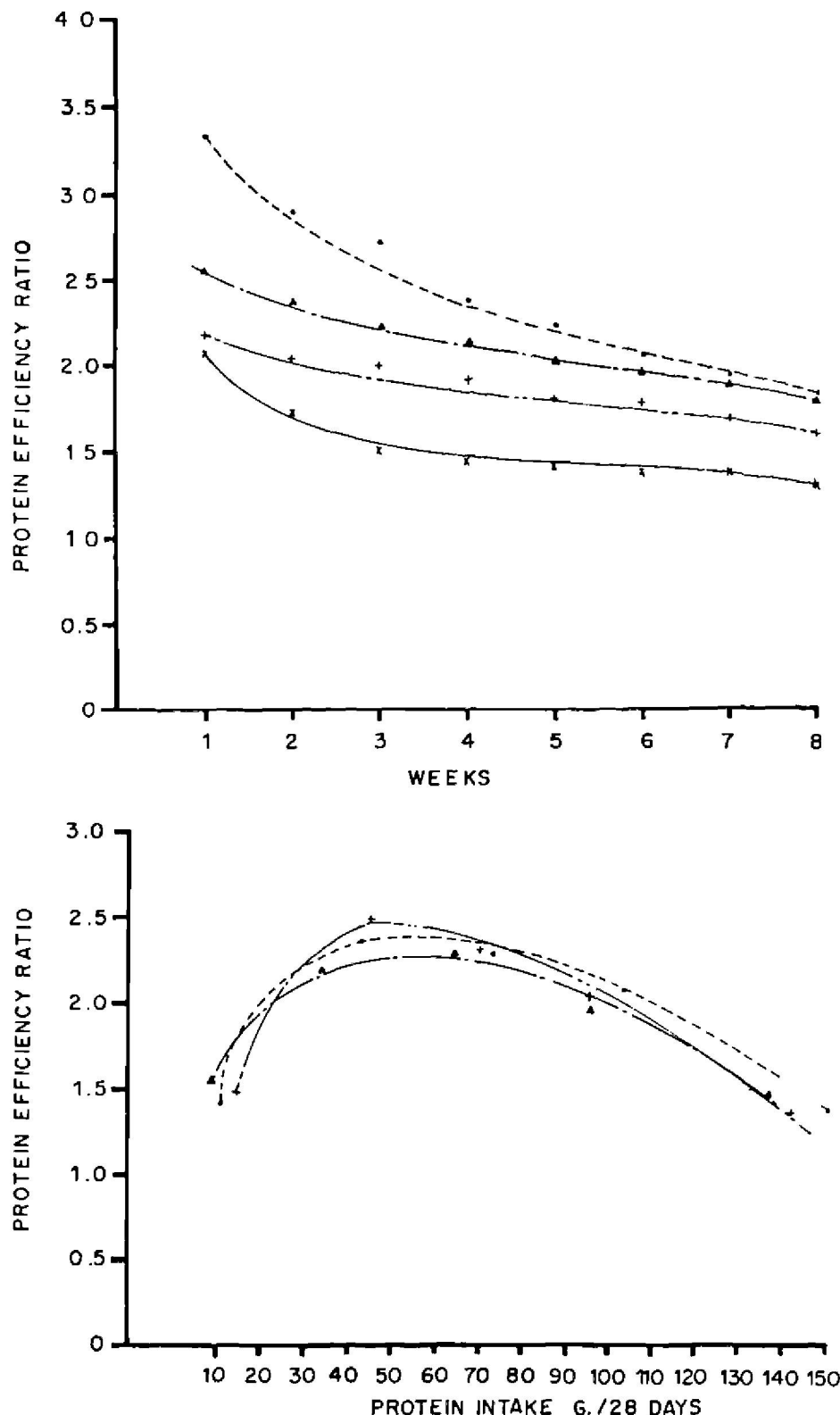


Fig. 1. Top, effect of time on protein efficiency ratio. Bottom, effect of protein intake on protein efficiency ratio. +—+ Soybean flour; - - - cottonseed flour; ▲—▲ peanut flour; ×—× sunflower flour.

Abb. 1. Oben: Wirkung der Versuchsdauer auf den Eiweißnutzwert. Unten: Wirkung der Eiweißzufuhr auf den Eiweißnutzwert. +—+ Soyamehl; - - - Baumwollsaamenmehl; ▲—▲ Erdnussmehl; ×—× Sonnenblumensamenöl.

Fig. 1. En haut, action du temps sur l'efficacité protéique de la ration. En bas, action de la quantité de protéines ingérées sur l'efficacité protéique de la ration. +—+ farine de soja; - - - farine de graines de coton; ▲—▲ farine de pistache; ×—× farine d'hélianthe.

Results

Fig. 1 gives the results on the effect of time and protein intake on PER. In all cases, PER decreased as the experimental period increased. Although not shown in the figure, the effect was more marked

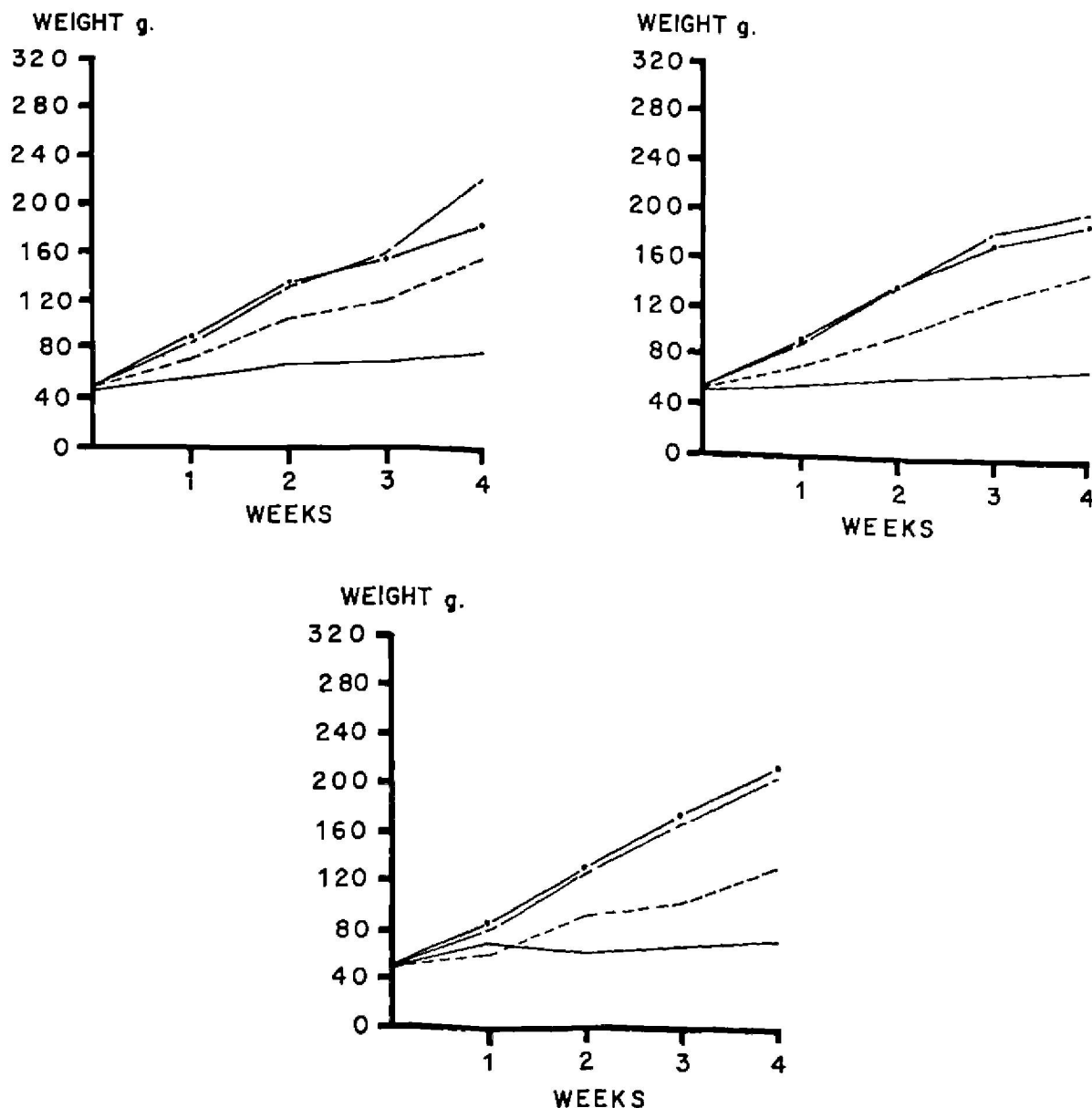


Fig. 2. Growth of rats fed three sources of protein at different levels of intake. Upper left, soybean flour; upper right, cottonseed flour; bottom, casein. — 5% protein, - - - - 10% protein, - - - 20% protein, — . — 30% protein.

Abb. 2. Wachstum von Ratten bei Fütterung mit dreierlei Proteinquellen mit verschiedenem Proteingehalt. Oben links: Soyamehl. Oben rechts: Baumwollsamenehl. Unten: Casein. — 5% Protein, - - - - 10% Protein, - - - 20% Protein, — . — 30% Protein.

Fig. 2. Croissance de rats nourris de 3 sources de protéines à différents pourcentages. En haut, à gauche, farine de soja; en haut, à droite, farine de graines de coton; en bas, caséine. — 5% de protéines, - - - - 10% de protéines, - - - 20% de protéines, — . — 30% de protéines.

for male than for female rats. A larger rate of decrease took place for cottonseed flour than for the other proteins studied. Although the maximum PER did not differ much between proteins, the amount

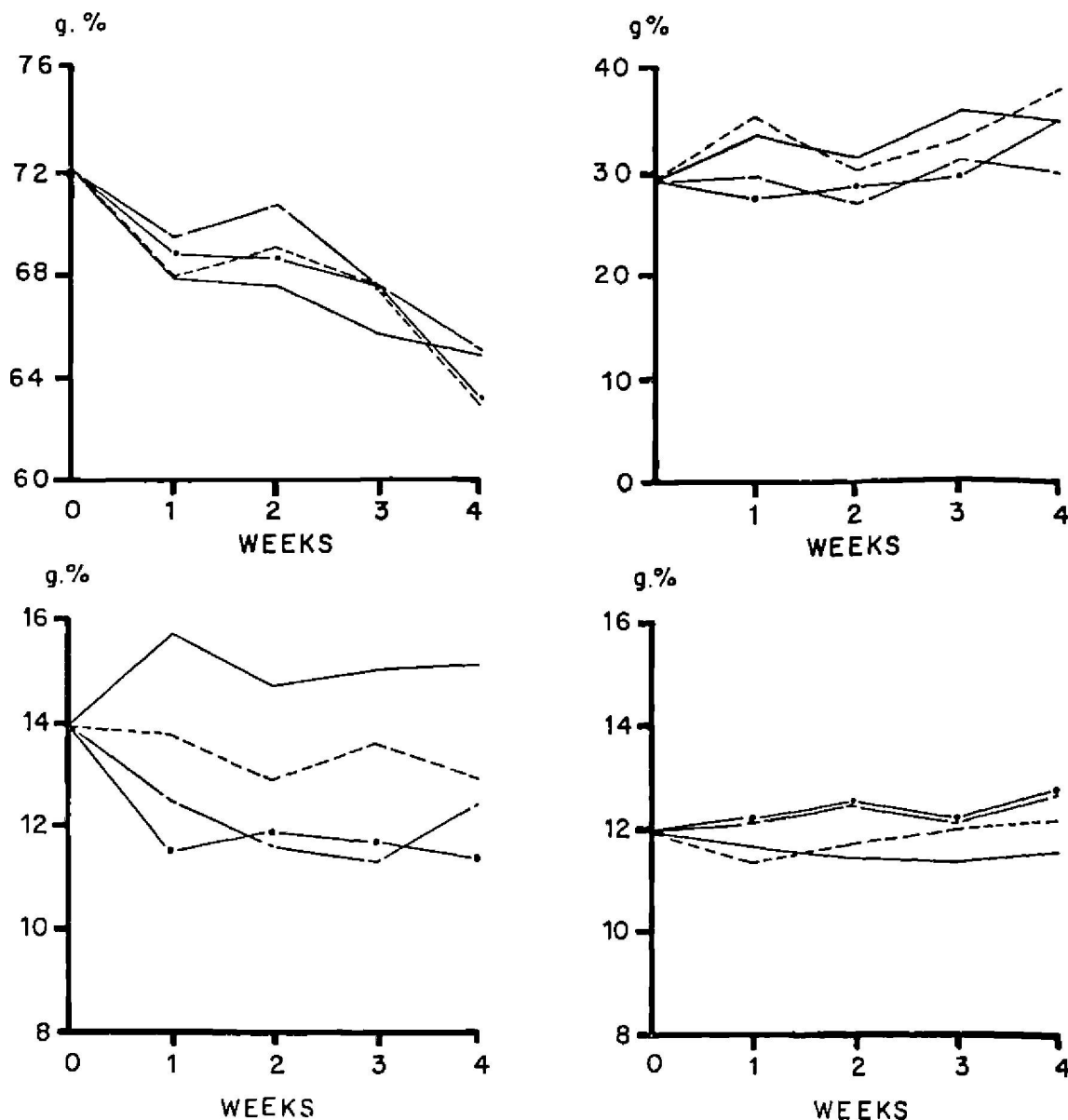


Fig. 3. Effect of time and protein level on carcass composition (casein diet). Upper left, water content; upper right, ether extract, % dry weight; bottom left, ash, % dry fat-free weight; bottom right, nitrogen, % dry fat-free weight. — 5% protein; - - - - 10% protein; - - - 20% protein; — . — 30% protein.

Abb. 3. Wirkung von Versuchsdauer und Proteingehalt auf die Zusammensetzung des eviszerierten Tieres (Caseinkost). Oben links: Wassergehalt, oben rechts: Ätherextrakt, % Trockengewicht, fettfrei; unten rechts: Stickstoff, % Trockengewicht, fettfrei. — 5% Protein, - - - - 10% Protein, - - - 20% Protein, — . — 30% Protein.

Fig. 3. Effet du temps et du pourcentage protéique sur la composition de la carcasse (régime à la caséine). En haut, à gauche, teneur en eau; en haut, à droite, extrait à l'éther, en % du poids sec; en bas, à gauche, cendres, en % du poids sec dégraissé; en bas, à droite, azote, en % du poids sec dégraissé. — 5% de protéines; - - - - 10% de protéines; - - - 20% de protéines; — . — 30% de protéines.

of protein needed for maximum PER was different. In the case of soybean flour, about 45 g protein/28 days gave maximum PER. In the case of cottonseed and peanut protein, the corresponding values were 55 and 60 g/28 days, respectively.

Fig. 2 shows the growth curves for soybean flour, cottonseed flour and casein; 20 or 30% of protein in the diet from any of these sources resulted in maximum growth.

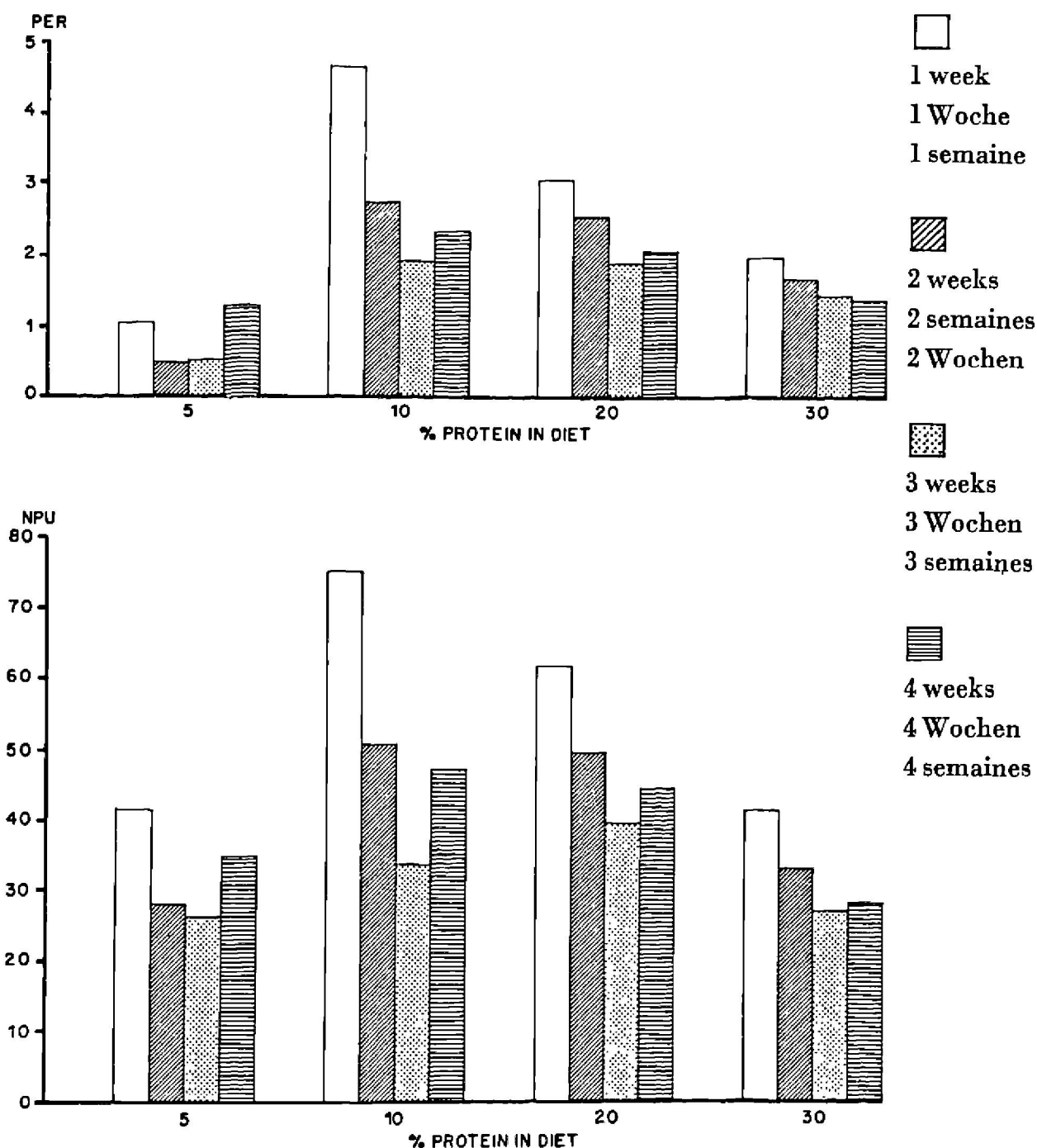


Fig. 4. NPU and PER at different levels of casein protein in the diet.

Abb. 4. Eiweißnutzwert und Wirkungsgrad bei verschiedenem Gehalt an Eiweiß (Casein) der Kost.

Fig. 4. NPU et PER à différents pourcentages de protéines, de caséine dans le régime.

The changes in carcass composition with respect to protein level in the diet and time are shown in Fig. 3 for casein. Moisture content decreased at all levels of casein feeding with respect to time. There

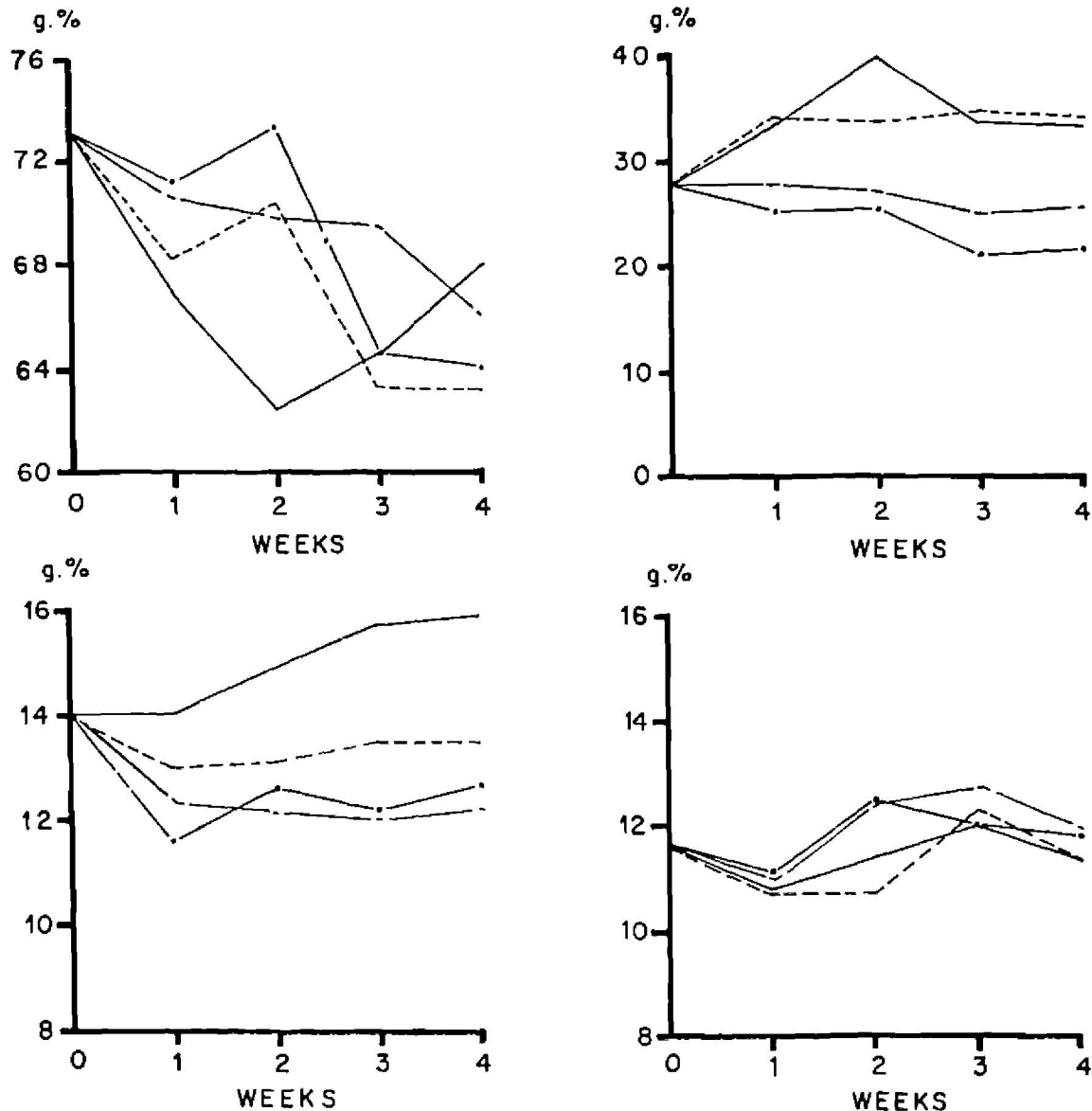


Fig. 5. Effect of time and protein level on carcass composition (soybean flour diet). Upper left, water content; upper right, ether extract, % dry weight; bottom left, ash, dry fat-free weight; bottom right, nitrogen, % dry fat-free weight. — 5% protein, - - - - 10% protein, - - - - 20% protein, — . — 30% protein.

Abb. 5. Wirkung von Versuchsdauer und Eiweißgehalt auf die Zusammensetzung des eviszerierten Körpers (Soyabohnenmehl). Oben links: Wassergehalt, oben rechts Ätherextrakt, % Trockengewicht, fettfrei; unten rechts Stickstoff, % Trockengewicht, fettfrei. — 5% Protein, - - - - 10% Protein, - - - - 20% Protein, — . — 30% Protein.

Fig. 5. Effet du temps et du pourcentage protéique sur la composition de la carcasse (régime à la farine de soja). En haut, à gauche, teneur en eau; en haut, à droite, extrait à l'éther, en % du poids sec; en bas, à gauche, cendres, en % du poids sec dégraissé; en bas, à droite, azote, en % du poids sec dégraissé. — 5% de protéines; - - - - 10% de protéines; - - - - 20% de protéines; — . — 30% de protéines.

was no appreciable change in percentage fat, ash or nitrogen content with respect to time; however, 20 and 30% protein in the diet resulted in higher levels of nitrogen in the carcass than 5 or 10%. On the other hand, the lower levels of protein resulted in higher levels of fat in the carcass than the higher levels of protein in the diet. The PER and NPU values for casein with respect to time and protein level are shown in Fig. 4. Protein efficiency ratio decreased with time when the

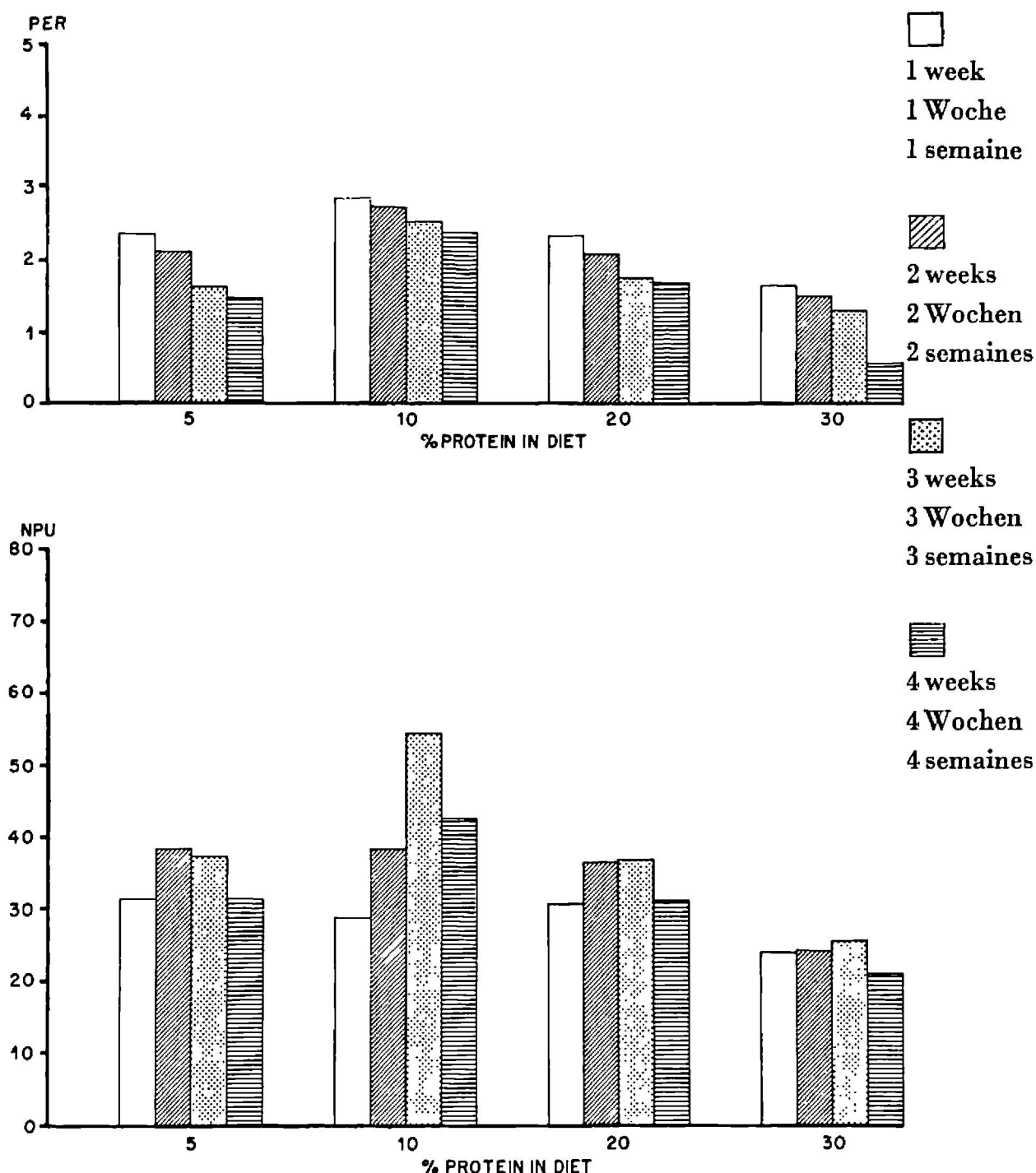


Fig. 6. NPU and PER at different levels of soybean protein in the diet.

Abb. 6. Eiweißnutzwert und Wirkungsgrad bei verschiedenem Eiweißgehalt (Soyabohnenmehl) der Kost.

Fig. 6. NPU et PER à différents pourcentages de protéines de soja dans le régime.

animals were fed 10, 20, and 30% protein. At the 5% level, there was a decrease during the second and third week and an increase

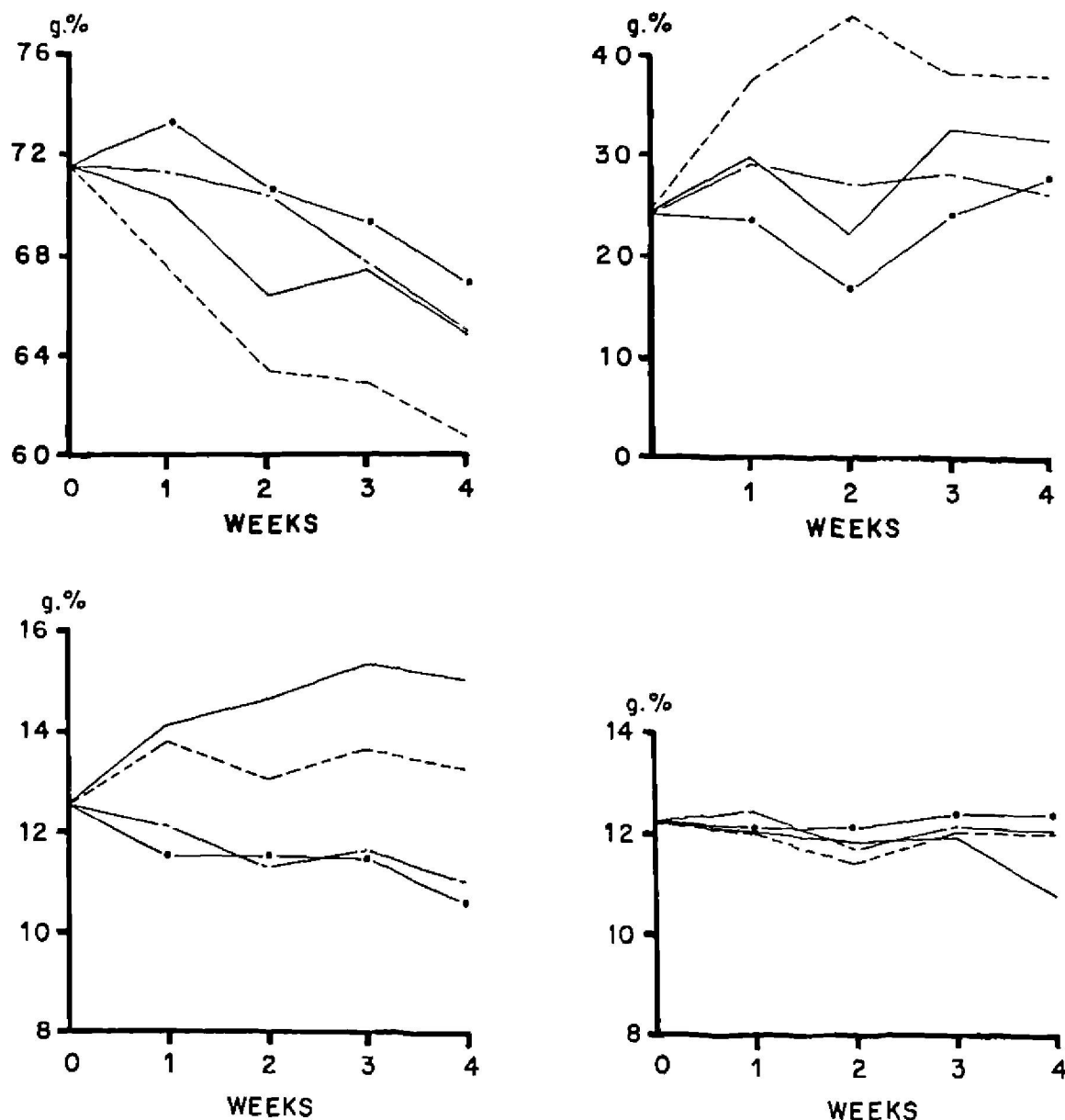


Fig. 7. Effect of time and cottonseed protein level in the diet on carcass composition. Upper left, water content; upper right, ether extract, % dry weight; bottom left, ash, % dry fat-free weight; bottom right, nitrogen, % dry fat-free weight. — 5% protein; - - - - 10% protein; - — - 20% protein; — . — 30% protein.

Abb. 7. Wirkung von Versuchsdauer und Eiweißgehalt (Baumwollsaamen) der Kost auf die Zusammensetzung des eviszerierten Körpers. Oben links: Wassergehalt; oben rechts Ätherextrakt, % Trockengewicht, fettfrei; unten rechts Stickstoff, % Trockengewicht, fettfrei. — 5% Protein, - - - - 10% Protein, - - — 20% Protein, — . — 30% Protein.

Fig. 7. Effet du temps et du pourcentage protéique sur la composition de la carcasse (régime aux graines de coton). En haut, à gauche, teneur en eau; en haut, à droite, extrait à l'éther, en % du poids sec; en bas, à gauche, cendres, en % du poids sec dégraissé; en bas, à droite, azote, en % du poids sec dégraissé. — 5% de protéines; - - - - 10% de protéines; - - — 20% de protéines; — . — 30% de protéines.

during the fourth week. Net protein utilization decreased for all protein levels from the first to the third week and an increase was observed during the last week. If NPU and PER are plotted against protein level, the curves for both are very similar with 10% protein in the diet giving in most cases maximum values.

The changes in carcass composition with respect to time and to protein level are shown in Fig. 5 for soybean flour. Moisture content decreased with respect to time for all protein levels in the diet. Fat and ash content were lower at the 20 and 30% protein level than at lower levels, while carcass nitrogen was higher at the higher protein intakes. Fig. 6 shows the NPU and PER values for soybean flour. PER decreased for all protein levels, but NPU tended to increase first and then decrease. When the values are plotted against protein level, the PER and NPU curves are similar with a maximum protein level of 10% in the diet. The results for the studies on carcass composition using cottonseed flour are shown in Fig. 7. Water content decreased at all protein levels with respect to time. Fat and ash content were lower for the higher protein diets and carcass nitrogen tended to be higher for the higher protein diets. Fig. 8 shows the PER and NPU values; PER increased slightly at two weeks and then decreased at 3 and 4 weeks. NPU increased at 2 weeks and decreased at 4 weeks; when the values are plotted against protein level the PER and the NPU curves are similar and maximum values were obtained at the 10% protein level in the diet.

Discussion

Results by several investigators [9] have indicated, as did those presented in this paper, that PER decreases with respect to time and that all proteins have a point where they are utilized with the greatest efficiency. This point of maximum utilization varies inversely with protein quality, the poorer the quality the more is needed for maximum PER [10]. As the animal matures, moisture content in the carcass decreases, as has been reported previously by *Widdowson et al.* [11]. Animals consuming the lower levels of protein had less carcass moisture and it decreased faster than higher levels of dietary protein. This is logical since upon protein deposition, water must be retained. It is interesting to see that carcass percentage nitrogen is very constant with respect to time, and this finding can be used to support

the calculation of NPU and NER (Nitrogen Efficiency Ratio) as described by *Bressani et al.* [12].

The level of protein at which maximum PER or NPU was obtained was 10%, which does not support optimum growth in rats, but higher levels resulted in decreased PER or NPU values since part of

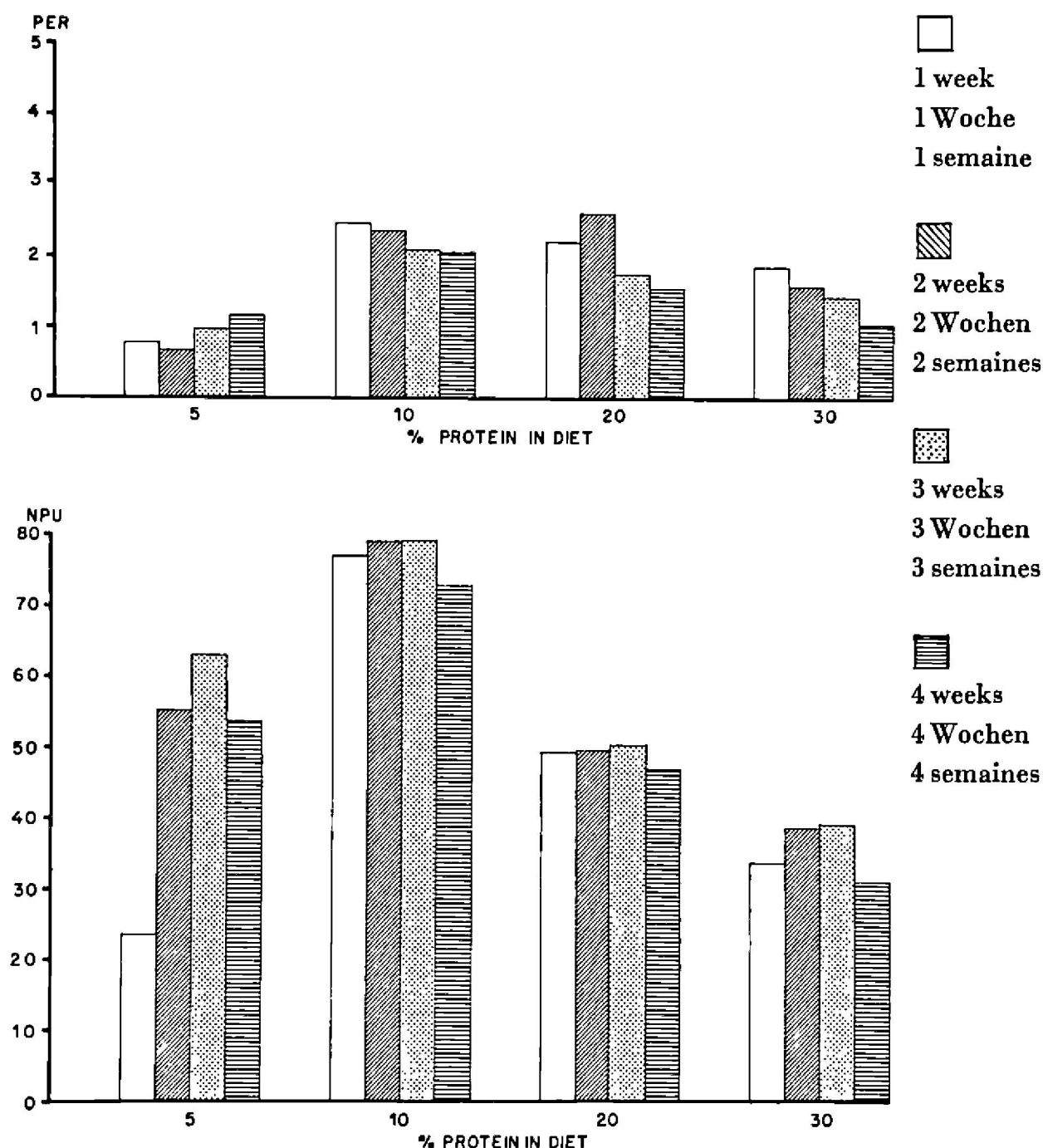


Fig. 8. NPU and PER at different levels of cottonseed protein in the diet.

Abb. 8. Eiweißnutzwert und Wirkungsgrad bei verschiedenem Gehalt an Baumwollsamenseitenprotein der Kost.

Fig. 8. NPU et PER à différents pourcentages de protéines de graines de coton dans le régime.

the protein would be utilized for energy purposes as well as for growth.

Net protein utilization values decreased with respect to time as did PER, and if NPU values are plotted against protein level in the diet, there is a maximum NPU value at 10% protein. However, as for PER, the amount of nitrogen required for NPU maximum values varies inversely with the quality of the protein, thus when the casein diet was fed, 2.73 g of nitrogen were needed to obtain optimum NPU values at two weeks, while 3.38 and 3.48 g of nitrogen were needed for cottonseed flour and soybean flour, respectively.

The decreasing values for both NPU and PER as time progressed suggest that for the former the standardized 10-day assay period probably gives maximum values, but for the latter, the 28-day assay period appears too long, and a shorter assay period would not only result in a saving of time, but would also increase the sensitivity of the method when used as a screening procedure for a series of proteins, since at that time differences between good and poor quality proteins would show at a maximum and the amounts of nitrogen needed for maintenance purposes would tend to be minimized.

Summary

Several experiments with rats were made to determine the effect of duration of test, protein level and protein quality on both NPU and PER values. The effect on carcass composition was also studied. The results indicated that NPU and PER were inversely proportional to both time and protein level. Two weeks and 10% of protein in the diet gave maximum values for both variables studied. In all cases, the water content of the carcass decreased with time, but increased with protein level. Fat and ash content were inversely proportional to nitrogen content of the diet, while carcass nitrogen paralleled nitrogen intake.

Zusammenfassung

Bei Ratten werden mehrere Versuche ausgeführt, um die Wirkung der Versuchsdauer, des Eiweißgehaltes und des biologischen Eiweißwertes auf Eiweißnutzwert (NPU) und Wirkungsgrad (PER) zu bestimmen. Auch die Zusammensetzung des Körpers wurde untersucht. Die Ergebnisse zeigten, daß NPU und PER umgekehrt proportional zu Zeit und Eiweißwert stehen. Zwei Wochen und 10% Eiweiß in der Kost ergeben Maximalwerte aller untersuchten Parameter. In allen Fällen fiel der Wassergehalt des Körpers mit der Zeit und stieg mit dem Eiweißgehalt der Kost. Fett- und Aschegehalt waren umgekehrt proportional zum Stickstoffgehalt der Kost; der Stickstoffgehalt des Körpers verhielt sich parallel der Stickstoffzufuhr.

Résumé

Plusieurs expériences ont eu pour but de déterminer sur le rat l'effet de la durée de l'essai, du taux protéique et de la qualité des protéines, sur les valeurs des deux paramètres: utilisation protéique nette et coefficient d'efficacité protéique. Les résultats montrent qu'ils sont tous deux inversement proportionnels au temps et aussi au taux protéique. Une durée de deux semaines et un pourcentage de 10% de protéines dans le régime donnent des valeurs maximum pour les deux variables étudiées. Dans tous les cas, la teneur en eau de la carcasse diminue en fonction du taux protéique. Les teneurs en graisse et en cendres sont inversement proportionnelles à la teneur en azote du régime, tandis que l'azote de la carcasse est proportionnel à l'ingéré azoté.

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