Turk J Vet Anim Sci
25 (2001) 149-154

In vitro Estimation of the Solubility of Dry Matter and Crude Protein of Wet Feed and Dry*

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Received: 13.04.1999

Abstract: Two experiments were conducted to determine the *in vitro* solubility of dry matter and crude protein of wet feed and dry feed of a commercial broiler diet. In the first experiment, to two g of feed samples of a commercial broiler pelleted diet was added 0-dry (80 g/kg water content) and 1.5 g water per g of feed (640 g/kg water content), and they were then incubated at one of two different levels of pepsin-HCl solutions, low (48 mg pepsin in 2 ml 0.1M HCl), or high (64 mg pepsin in 3 ml 0.1M HCl) for 0, 15, 30, 60, 120, 180, and 240 minutes at 42ºC. In the second experiment, to 2 g of a commercial broiler feed was added 0-dry (80 g/kg water content), 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 1.8, and 2.0 g water per g of dry feed (240, 340, 430, 480, 540, 580, 630, and 700 g/kg water content, respectively). To each feed sample was also added 64 mg pepsin with 3 ml-HCl (the high level from experiment 1), and then incubated for 10, 20, 30, 60, 180 and 240 minutes at 42ºC. After filtration and drying processes, dry matter and crude protein analyses were done for each sample.

In experiment 1, wetting the feed with 1.5 g water per g feed followed by incubation in pepsin-HCl solution increased both dry matter solubility and crude protein solubility. There were also higher solubilities of feed nutrient with a high concentration of pepsin-HCL with a than low concentration of pepsin-HCL.

In experiment 2, dry feed samples (80 g/kg water content) and feed samples with 240 and 340 g/kg water content had similar nutrient solubility, but their solubility values were significantly ($P<0.05$) lower than those of wet feed samples with 540, 580, 630 and 700 g/kg water content. Feed samples with 630 and 700 g/kg water content had high solubility values throughout the incubation periods.

The results revealed that increased nutrient solubility might just as easily have been achieved by the addition of a larger volume of liquid to dry feed because additional water in wet feed samples was the only difference between these and the dry feed samples.

Key Words: wet feed, dry matter, crude protein, in vitro solubilization, pepsin, HCl

Islak ve Kuru Yemlerde Kuru Madde ve Ham Protein Solubilizasyonunun *in vitro* Tespiti

Özet: Kuru ve ıslatılmış broyler yemlerinde kuru madde ve ham proteinin in vitro solubilizasyon tespiti için iki deneme yapılmıştır. Deneme 1 de, havada kuru (yaklaşık 80 g/kg su içerikli) ve 640 g/kg su içerikli ıslak yem (g kuru yeme 1,5 g su ilave edilmiş) numulerinden 2 gram yem düşük (48 mg pepsin-2 ml 0,1 M HCl) ve yüksek (64 mg pepsin-3 ml 0,1 M HCl) konsantrasyonlu pepsin-HCl çözeltilerinde 42ºC sıcaklıkta, 0, 15, 30, 60, 120, 180 ve 240 dakika sürelerde inkübasyona tabi tutulmuşlardır. Deneme 2 de, sırasıyla 0; 0,2; 0,4; 0,6; 0,8; 1,0; 1,5; 1,8; ve 2,0 g su/g yem oranında su katılarak hazırlanmış 80, 240, 340, 430,480, 540, 580, 630 ve 700 g/kg su içerikli yem numuneleri sadece deneme 1'deki yüksek konsantrasyonlu-68 mg pepsin-3 ml HCl-çözeltisinde 42 derece sıcaklıkta 10, 20, 30, 60, 180 ve 240 dakika tutulmuşlardır. Filtre ve kurutma işlemlerinden sonra yem numunelerinde kuru madde ve ham protein analiz edilmiştir.

Deneme 1 de her gram yem numunelerine 1,5 gram su ilave edilip pepsin-HCl çözeltisinde *in vitro* inkübasyona tabi tutulması hem kuru madde ve hem de ham protein solubilizasyonunu artırmıştır. Bu denemede ayrıca yüksek konsantrasyonlu pepsin-HCl çözeltisi ile inkübe edilen yem numuneleri düşük konsantrasyonda inkübe edilmiş numunelerine nazaran daha yüksek solubilizasyon değerleri vermiştir.

Deneme 2 de, kuru yem numuneleri ile 240 ve 340 g/kg su içerikli numuneler benzer solubilizasyon değerleri vermiş, fakat bu değerlerin 540, 580, 630 ve 700 g/kg su içerikli ıslak yem numunelerinkinden önemli ölçüde ($P<0,05$) düşük olduğu tespit edilmiştir.

Deneme sonuçlarına göre,ıslak yem numuneleri ile kuru yem numuneleri arasında gözlenen tek farklılığın sadece ıslak yem örneklerine dışarıdan katılan ek su olması, ıslak yem numunelerinde görülen yüksek besin madde solubilizasyonunun sadece kuru yeme büyük oranda su ilavesi ile gerçekleşmiş olabileceğini ortaya koymaktadır.

Anahtar Sözcükler: ıslak yem, kuru madde, ham protein, *in vitro* solubilizasyon, pepsin, HCl

* This study is a part of phD thesis and was carried out of Leeds University, UK.

Introduction

Increased weight gain and improved feed conversion efficiency (FCE) in broiler chickens fed with wet feed in comparison with the same diet without addition of water have previously been reported (1). The significant improvements in feed intake and weight gain, but not in the FCE, of broiler chickens fed with wet feeds of cereal-based diets are also of great interest in poultry production (2, 3, 4, 5, 6). The results of the above studies implied that improved performance with wet feeding is likely to be associated with an increase in the rate of digestion and absorption of nutrients in the digestive tract. A remarkable, but not significant, increase in the apparent retention of dry matter and crude protein of feed fed to birds in wet form has also been reported (1).

In vitro estimation of feed dry matter and crude protein was previously studied with different pepsin-HCl concentrations, and the results showed that increasing both pepsin and HCl concentrations in a digestion solution caused remarkable increases in the soluble or digestible amount of feed dry matter and crude protein (7, 8).

Mixing feed with water can change its texture from a basic structure of feed particles combined together in the original dry diet to a moisturised structure where the feed particles become dispersed (9).

The hypothesis tested here is that wetting feed could result in a higher solubility of feed nutrients, compared to the same feed without added water, and this may lead to a further increase in the rate of nutrient digestion once wet feed mixes with digestive enzymes and secretions (10).

For this purpose we conducted two experiments in which the nutrient solubility of feed samples with different water contents was determined using an in vitro method in which feed samples were incubated in a water bath with different concentrations of pepsin-HCl solution for several minutes.

Materials and Methods

Two experiments were conducted to test the effects of water on the in vitro solubility of dry matter and crude protein of the samples of wet and dry feed.

In experiment 1, to two g of feed samples of a commercial broiler pelleted diet was added 0-dry (80 g/kg water content) and 1.5 g water per g of feed (640 g/kg water content). The samples were then incubated at two different levels of pepsin-HCl solution (pepsin obtained from Sigma cat. no. P7000, 1:10.000, in powder form with an activity of 800-2500 units per mg): low (48 mg pepsin in 2 ml 0.1M HCl) or high (64 mg pepsin in 3 ml 0.1M HCl) for 0, 15, 30, 60, 120, 180, and 240 minutes in a water bath. Wet feed samples were prepared by adding distilled water 10 minutes before the digestion solution was added, in order to give the feed samples enough time to absorb all the water. However, wet feed samples were in a greater volume of liquid per g dry matter compared with dry feed. The feed was a commercial broiler finisher pellet diet obtained from Dalgety Agriculture Ltd, Bristol, UK (crude protein, 195.0 g/kg; crude fibre, 37.5 g/kg; ME, 13.4 MJ/kg).

In experiment 2, to two replicates of 2 g of a commercial broiler feed of the same origin as in experiment 1 was added 0-dry (80 g/kg water content), 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 1.8, and 2.0 g water per g of dry feed (240, 340, 430, 480, 540, 580, 630, and 700 g/kg water content, respectively). To each feed sample was also added 64 mg pepsin with 3 ml-HCl (the high level from experiment 1). The samples were then incubated for 10, 20, 30, 60, 180 and 240 minutes in a water bath at 42ºC and 150 cpm (cycles per minute).

Determination of nutrient solubility of feed samples after each incubation time was carried out using the same in vitro procedure as in experiment 1. Experiment 2 was of a factorial design with 9 different feed samples x 6 incubation times.

In vitro Solubility Procedure

The initial studies with this procedure produced variable results, and further trials were therefore designed to study all conditions, including amounts of sample, types of incubation devices, concentrations of pepsin and filtration methods affecting the accuracy of the method. The final in vitro solubility procedure was as follows:

To two replicates of a 2 g sample from feed was added 0-dry or 1.3 g /g feed water just 10 min before the addition of digestion solution. To samples of both dry and wet feed was added 48 mg powdered pepsin in 2 ml of 0.1 M. HCl, or 64 mg powdered pepsin in 3 ml of 0.1 M HCl, followed by incubation in an agitating water bath for 0, 15, 30, 60, 120, 180, and 240 minutes in experiment

1, and 10, 20, 30, 60, 180 and 240 minutes in experiment 2 at 42ºC (deep body temperature in chickens) and 140 cpm (cycle per min). After the addition of digestion solution 2 or 3 ml (referring HCl), dry feed samples would differ in total liquid volume from wet feed in which the activity of the pepsin enzyme would be more diluted than in its dry counterpart although both feed samples contain the same amount of pepsin enzyme per g dry matter, assuming that the gastric pepsin level, although perhaps not its activity, would be similar in birds whether they were fed with dry or wet feeds. After each incubation time the samples, in 25 ml centrifuge tubes, were removed from the agitating water bath, and their contents were slowly filtered through filter paper (Whatman no. 541) in a continuous small stream, thus avoiding unnecessary agitation. Each filter paper was dried and cooled and its weight recorded before incubation. After all the liquid had been passed through the filter paper, it was placed in an evaporating dish to dry in an oven at 105ºC overnight and then weighed again as before.

The solubility of dry matter of samples was determined as follows:

$$\mathbf{SDM}, (g/kg) = (DM^{A} - DM^{B}) / (DM^{A}) \times 1000$$

where **SDM** is the solubility of dry matter (g/kg); DM^{A} is a g dry matter of the sample and DM^{B} is a g dry matter of undigested residue, expressed to be as g soluble dry matter per 1000 g of feed dry matter.

Indigestible protein in the total residue was determined by transferring the filter paper containing the residue directly to a Kjeldahl flask using the AOAC (1984) procedure (11). After a blank determination on one sheet of filter paper and determination of protein content of the original diet, the in vitro protein digestibility was calculated as follows:

$$\mathbf{SCP}, (g/kg) = (\mathbf{CP\ feed} - \mathbf{CP\ undigested}) / (\mathbf{CP\ feed}) \times 1000$$

where **SCP** is solubility of crude protein (g/kg), **CP feed** is g, crude protein in 2-gram sample, and **CP undigested** is g, crude protein in residue, expressed as g soluble crude protein per 1000 g of feed crude protein.

A general linear model (GLM) with repeated measures (incubation times) was used to determine the significance of the main effects of physical form of the feed and levels of pepsin-HCl solution, and of their interactions on the parameters using the MINITAB (1993) statistical analysis program (12). Appropriate means were separated using the multiple range test (13).

Results

In experiment 1, wetting feed with water significantly ($P<0.05$) increased the amount of soluble dry matter and crude protein of feed, at both levels of pepsin-HCl (P-HCl) (Table 1).

Table 1. Treatment means and results of statistical analyses for in vitro solubility of dry matter (SDM, g/kg DM) and solubility of crude protein (SCP, g/kg DM) of wet and dry feed samples (Exp. 1).

Wetness	SDM ,	SCP
Dry feed	219.5[a]	370.9[a]
Wet feed	263.7[b]	431.7[b]
SEM	2.7	3.6
Level of pepsin-HCL		
Low	234.8[a]	379.0[a]
High	248.5[b]	424.0[b]
SEM	2.7	3.6
Significance of treatment effects		
Wetness (W)	***	***
Level of Pepsin-HCl (P)	**	***
Incubation time (T)	***	***
W by P	NS	NS
W by T	NS	NS
P by T	NS	***
W by P by T	NS	**

[ab,] letter within the same column with dissimilar superscripts indicates significant ($P<0.01$) differences between treatments (mean SEM, standard error between means). NS, not significant; *$P<0.05$; **$P<0.01$; ***$P<0.001$.

The solubility of dry matter and crude protein of feed samples significantly ($P<0.05$) varied between the two levels of P-HCl. The effect of incubation time was also significant ($P<0.05$) on the solubility of feed nutrients, as increasing incubation time increases nutrient solubility.

The interaction between wetness and levels of P-HCl solution did not have a significant effect on the solubility of feed nutrients. Higher solubility of dry matter and crude protein were seen with wet feed samples at "0" time of incubation than with to dry feed samples, although there was a significant difference in both dry

matter and crude protein solubility between dry feed and wet feed samples in all incubation periods. The interaction between the effects of wetness and incubation time was also not significant (Figure 1).

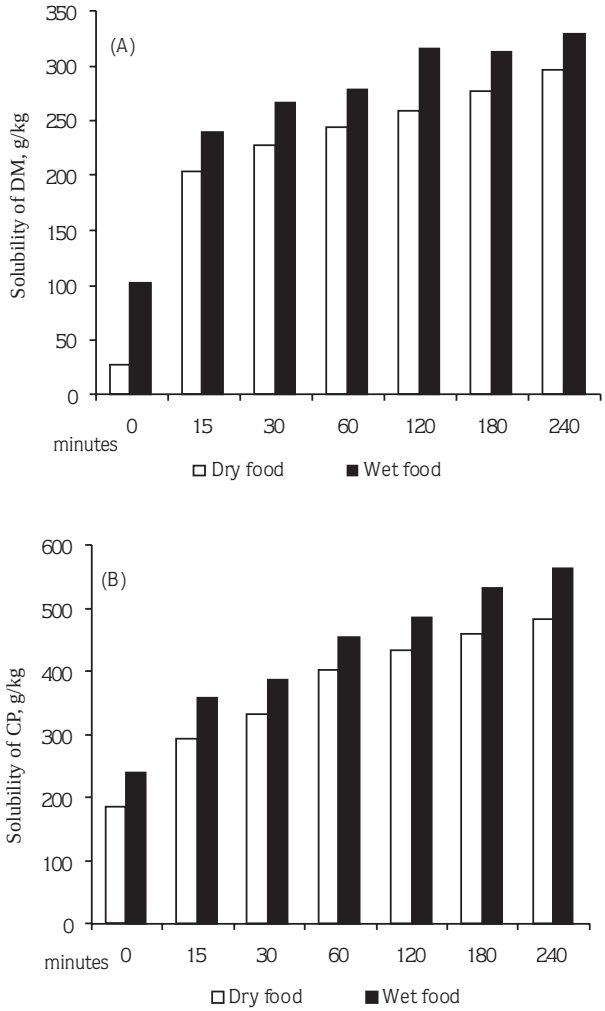

Figure 1. Solubility of dry matter (**A**) and crude protein (**B**) of wet and dry feed samples with P-HCl solution in various incubation periods (Exp. 1).

In this experiment, 2 replicates of dry and wet feed samples were left at room temperature for 15 minutes without addition of pepsin-acid solution, and then dry matter solubility was measured for both feed samples. Wet samples had an average of 170 g/kg dry matter solubility, while that of dry samples was 90 g/kg. The other 2-way and 3-way interaction effects in experiment 1 were not found to be significant on the solubility of dry matter of feed samples, but the interaction effects of P by T and W by P by T were significant on the soluble amount of crude protein of feed samples. It is difficult to interpret such 2-way and 3-way interaction effects.

In experiment 2, increasing the water content of feed from 80 g/kg water (dry form) to 700 g/kg (a semi-liquid form), by adding water directly, substantially increased both in vitro dry matter and crude protein solubility of feed samples (Table 2).

Feed samples with 80 g/kg (dry form), 240 and 340 g/kg water content had similar nutrient solubility, but their solubility values were significantly ($P<0.05$) lower than of feed samples with 540, 580, 630 and 700 g/kg water content. Higher solubility values were obtained from feed samples with 630 and 700 g/kg water content throughout the incubation periods. The results clearly showed that wetting positively affected nutrient solubility of poultry feeds such that the amount of water needed is very critical to obtain significant increases in nutrient solubility. It was obvious that wet feed samples with 630 and 700 g/kg water content became highly soluble within 10 to 30 minutes of incubation in a P-HCl solution (Figure 2).

Table 2. Effects of diets with varying water content on the mean values of in vitro solubility of dry matter (SDM, g/kg) and of crude protein (SCP, g/kg) in Exp. 2.

Water content (g/kg)	SDM	SCP
80 (Dry)	204.5^{a}	308.2^{a}
240 (Wet)	205.4^{a}	317.6^{ab}
340 (Wet)	210.8^{a}	335.4^{abc}
430 Wet)	213.2^{a}	344.6^{abc}
480 (Wet)	222.5^{a}	356.7^{bc}
540 (Wet)	227.0^{ab}	367.8^{c}
580 (Wet)	231.8^{b}	378.0^{cd}
630 (Wet)	253.2^{b}	411.1^{d}
700 (Wet)	294.9^{c}	458.4^{e}
SEM	1.73	3.76
Significance of treatment effects		
Water (W)	***	***
Time (T)	***	***
W by T	***	***

$^{a, b, c}$ letters within the same column with dissimilar superscripts indicate significant ($P<0.01$) differences between treatments (mean SEM). ***$P<0.001$.

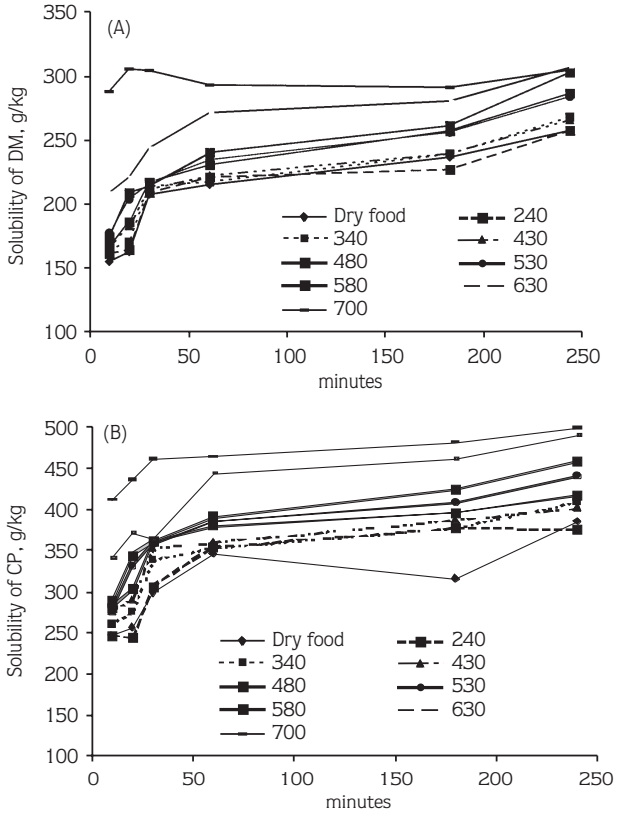

Figure 2. Effects of varying additions of water to feed on the in vitro solubility of dry matter (**A**) and crude protein (**B**) of a commercial broiler diet (Exp. 2).

Discussion

The in vitro solubility values obtained from the present experiments were found to be similar to those obtained previously (7 and 8). Increasing the level of P-HCl acid from 48 mg to 64 mg without regard for the effect of water addition resulted in an increase in the nutrient solubility of feed samples in experiment 1 (Table 3.1), and this was in agreement with the results of previous studies (7), in which pepsin concentrations were increased from 270 units/10 ml HCl to 580 or 1140 units/10 ml HCl. There was also demonstrated to be a significant increase in the digestibility of DM and CP when the pepsin concentration was increased from 1 to 2 mg in 10 ml HCl solution (8), similar to the present case.

The results of the present experiments are not comparable to those digestibility results obtained in animal experiments (1) since the in vitro conditions in our experiments to test the solubility of feed nutrients do not completely represent the in vivo digestibility conditions in the birds. However, a recent study undertaken to determine the in vivo nutrient digestibility of wet feed indicates that increasing the solubility of dry feed with additional water may result in a high rate of digesta passage through the gut, resulting in less time for digestion to occur (10).

Experiment 1 also showed an increase in the nutrient solubility of feed mixed with water and showed that wetted feed, without incubation with enzyme-acid solution, had higher solubility of dry matter (170 g/1000 g), within 15 minutes of wetting, than dry feed (90 g/1000 g). One may question whether or not the level of P-HCl in wet feed samples is more dilute than that in dry feed during incubation due to the additional water in wet feed samples.

However, the same quantity of P-HCl on the basis of each g dry matter was added to both feed samples. Therefore, the additional water in the wet feed samples is the only difference between them and the dry feed samples, and this suggests that the effect might just as easily have been achieved by the addition of a larger volume of liquid to dry feed.

There can therefore be said to be a physical or an enzymatic solubilisation action of water on the feed particles when feed is about to given to birds, since the experimental findings given below support this claim.

Several studies have indicated that wetting feed, particularly bird feed based on cereal grains, causes a significant reduction in digesta viscosity (2, 4, 5, 6, 9) and in the number of epithelial cells being proliferated from the crypts to the surface of epithelium in the gut (3 and 9) as an indication of rapid penetration of digestive secretion into feed particles and an ease of digestion. There is thus a considerable saving the epithelial cells in terms of energy and protein. This may be due to the effect of the water added to feed, since the feed remains in front of the birds for at least 12 hours, during which time the feed may start some enzymatic action. In confirmation of this, some preliminary observations have shown that feed pH from the start of feeding until the finishing of feeding drastically decreases, from pH 6.5 to pH 4.3, suggesting some pre-ingestion enzymatic actions within the feed (unpublished results) (14).

In light of the above findings, it can be concluded that wetting feed may enhance the nutrient solubility, stimulating a pre-enzymatic digestion to occur before the ingestion of feed, since wetting reduces the pH of feed (14), viscosity of digesta (2, 4, 5, 6, 9) and epithelial cell sloughing (3 and 9).

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