Interactions of Various Supplies of Isoleucine, Valine, Leucine and Tryptophan on the Performance of Laying Hens

S. Peganova and K. Eder[1]

Institut für Ernährungswissenschaften, Martin-Luther-Universität Halle-Wittenberg, Emil-Abderhalden-Strasse 26, D-06108 Halle/Saale, Germany

ABSTRACT The present study was undertaken to investigate the interactions among the supplies of isoleucine, leucine, valine, and tryptophan in laying hens. A three-factor trial was conducted with laying hens in which the dietary concentrations of isoleucine (5.7, 8.0, and 11.5 g/kg), valine and leucine (6.3 and 7.2 g/kg and 10.1 and 11.5 g/kg, respectively), and tryptophan (1.5 and 2.4 g/kg) were varied. At the lowest concentration of valine + leucine, an increase in dietary isoleucine concentration led to a dose-dependent reduction in feed consumption, daily egg mass, and body weight gain and an increase of the isoleucine concentration in plasma. At a high dietary concentration of valine + leucine, excess dietary isoleucine concentration caused only a weak depression of performance parameters; the isoleucine concentration in plasma was independent of the dietary isoleucine concentration. Increasing the dietary tryptophan concentration did not influence the effect of an excessive dietary isoleucine concentration on performance parameters. Increasing the tryptophan concentration from 1.5 to 2.4 g/kg diet did, however, lead to a significant increase in feed consumption, irrespective of the supply of isoleucine, valine, and leucine. In conclusion, our study demonstrates that the supply of valine + leucine influenced the effects of excess dietary isoleucine in laying hens, whereas the supply with tryptophan did not.

(*Key words*: laying hen, isoleucine, valine, leucine, interactions)

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INTRODUCTION

A recent study has shown that the margin between requirement and excess of the amino acid isoleucine is very narrow in laying hens (Peganova and Eder, 2002). For maximum daily egg mass from laying hens, a dietary isoleucine concentration of 4.0 g/kg is necessary. On the other hand, increasing the concentration slightly to 8.0 g/kg diet leads to a reduction in body weight; at 10.0 g/kg, feed consumption and daily egg mass are reduced as well. The performance-depressant effect of excess dietary isoleucine might be due to antagonisms between this and the two other branched-chain amino acids, valine and leucine. The three structurally similar branched-chain amino acids (valine, leucine, and isoleucine) share common systems for transport through cellular membranes and use the same enzymes for degradation (Harper, 1984). Antagonisms among these three amino acids have already been demonstrated in several species such as rats, pigs, turkeys, and broilers (Sauberlich, 1961; Allen and Baker, 1972; Oestemer et al., 1973; Tuttle and Balloun, 1976; Smith and Austic, 1978; Taylor et al., 1984). It has been shown in broiler chicks that increasing the dietary concentration of leucine alleviates the performance-depressant effect of an excessive isoleucine supply (Burnham et al., 1991). Similar interactions have not been studied in laying hens. Isoleucine and other large neutral amino acids also compete with tryptophan for transfer into the brain across the blood-brain barrier (Wurtman, 1980; Tackman et al., 1990). Tryptophan plays an important role in the brain as a precursor of the neurotransmitter serotonin, which has a major effect on the feeding behavior of animals among its many functions (Blundell and Latham, 1978; Tackman et al., 1990; Mullen and Martin, 1992). As excess dietary isoleucine was associated with a marked reduction in feed consumption, the obvious assumption was that effects of excess dietary isoleucine might also be due to a secondary tryptophan deficiency in the brain. The present study, designed as a three-factor experiment, was undertaken to investigate the interactions among the supplies of isoleucine, valine + leucine, and tryptophan. In particular, we wanted to find out whether the performance depression caused by an excessive supply of isoleucine could be alleviated by increasing the supply of the other two branched-chain amino acids or increasing the supply of tryptophan. We

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[1]To whom correspondence should be addressed: eder@landw.uni-halle.de.

Abbreviation Key: BCKA = branched-chain α-keto acid.

TABLE 1. Composition of the basal experimental diet

Ingredient	Amount
	(g/kg)
Wheat	300
Barley	336
Peas	137
Cellulose	41
Soybean oil	54
Calcium carbonate	85
Calcium phosphate	15
Vitamin and mineral premix[1]	10
Salt	1.5
L-Lysine-HCl	2.5
DL-Methionine	1.9
L-Threonine	1.0
L-Tryptophan	0.3
L-Isoleucine	1.9
L-Valine	1.5
L-Aspartic acid	6.5
L-Glutamic acid	6.6
Analysis (g/kg)	
Crude protein	131
Methionine	3.7
Methionine + cysteine	6.1
Lysine	6.1
Tryptophan	1.5
Threonine	4.2
Isoleucine	5.7
Valine	6.3
Leucine	7.2
Calcium	37.0
Total phosphorus	6.9
Energy (MJ ME/kg), calculated[2]	11.44

[1]Supplied per kilogram of diet: calcium, 1.70 g; sodium, 0.80 g; vitamin A, 12,000 IU; cholecalciferol, 2,500 IU; DL-α-tocoopherol acetate, 20 mg; thiamine, 5 mg; riboflavine, 3 mg; pyridoxine, 3 mg; vitamin B_{12}, 20 μg; vitamin K_3, 1.2 mg; pantothenic acid, 8 mg; niacin, 30 mg; folic acid, 0.5 mg; choline chloride 150 mg; iron, 25 mg; zinc, 60 mg, manganese, 100 mg; copper, 5 mg; cobalt, 0.1 mg; iodine, 1 mg; selenium, 0.2 mg.

[2]Calculated according to data provided by Jahrbuch für die Geflügelwirtschaft (2000).

proposed to determine feed consumption, other performance parameters, and the concentrations of free amino acids in the blood plasma.

MATERIALS AND METHODS

An experiment was conducted with 144 Lohmann Brown layers from 25 to 28 wk of age. The laying hens were assigned to 12 treatment groups. We intended to use a diet in which the concentrations of branched-chain amino acids and tryptophan were in accordance with the recommendations of the German Nutrition Society (Gesellschaft für Ernährungsphysiologie, 1999). To avoid an excess concentration of dietary leucine, a basal diet with low protein was used. The basal diet was composed primarily of cereal and peas and contained 11.4 MJ ME/kg (Table 1). The basal dietary concentrations of isoleucine, valine, leucine, and tryptophan were 5.7, 6.3, 7.2, and 1.5 g/kg, respectively. In accordance with a three-factor trial design, the dietary concentrations of (I) isoleucine, (II) valine + leucine, and (III) tryptophan were varied. The dietary isoleucine concentrations were 5.7, 8.0, or 11.5 g/kg diet; the dietary concentrations of valine and leucine were 6.3 and 7.2 g/kg or 10.1 and 11.5 g/kg, respectively. The dietary concentration of tryptophan was 1.5 or 2.4 g/kg. The concentrations of those amino acids in the diets were varied by supplementing the basal diets individually with L-isoleucine, L-valine, L-leucine, or L-tryptophan.[2] The purity of those amino acids was at least 98%. The concentrations of the other essential amino acids were adjusted to an adequate level as recommended by Gesellschaft für Ernährungsphysiologie (1999) by supplementation with synthetic amino acids.

The hens were maintained one bird per cage in an environmentally controlled room at 18 C. Lighting was 14 h daily at 20 to 30 lx. Feed (in ground form) and water (via nipple drinkers) were available ad libitum. Because our prestudies showed that feed consumption and daily egg mass of laying hens respond quickly to amino acid antagonism in the diet, this experiment was restricted to 3 wk. All procedures followed established guidelines for the care and handling of animals and were approved by the veterinary council of Saxony-Anhalt.

The following data were recorded: BW at the start and end of the trial, feed consumption weekly, and number of eggs daily. Egg weight was determined on two eggs from each hen at the end of each week. At the end of the experiment, 4 h after feed withdrawal, blood samples were drawn from the vena jugularis from each bird to determine the concentrations of free amino acids in the blood (Kirchgessner et al., 1995).

The crude nutrient concentrations of the diets were analyzed according to official VDLUFA methods (Naumann and Bassler, 1993). Concentrations of amino acids were determined by hydrolyzing the diets with 6 *N* hydrochloric acid; the pH of the hydrolysate was adjusted to 2.2. Amino acids were separated and quantified by ion exchange chromatography in an amino acid analyzer[3] using a special separating column with cation exchanger resin.[4] The tryptophan concentration of the diets was determined by reverse-phase-HPLC[5] (Fontaine et al., 1998).

The determination of free amino acids in blood plasma was performed with the same amino acid analyzer as used for amino acid analysis of the diets. After precipitation of the plasma-protein compounds with 10% sulfosalicylic acid, samples were centrifuged (15,600 × *g*), pH of the solution adjusted to 2.2 by addition of a dilution buffer, and samples were applied to a special polyether ether ketone separating column with cation exchanger resin.[4]

The statistical analysis of the data was performed with the software Statistica for Windows (StatSoft, Inc., 2000). The data were tested for normal distribution and homogeneity of the variances. Data were evaluated by three-way analysis of variance with the factors isoleucine, valine + leucine, and tryptophan and the interactions of these

[2]Lohmann Animal Health, Cuxhaven, Germany.
[3]Biotronik LC 3000, Eppendorf, Hamburg, Germany.
[4]Laborservice Onken, Gruendau, Germany.
[5]Hewlett Packard HPLC system, Palo Alto, CA, equipped with an RP-18-e column (5-μm particle size, 250 × 4 mm).

TABLE 2. Performance of laying hens receiving diets with various concentrations of isoleucine, valine + leucine, and tryptophan from 25 to 28 wk of age[1]

Dietary treatment								
Isoleucine (g/kg)	Valine/leucine (g/kg)	Tryptophan (g/kg)	Feed consumption (g/hen/d)	Egg production (%)	Egg weight (g)	Daily egg mass (g/hen)	Feed/egg mass (g/g)	Body weight change (g)
5.7	6.3/7.2	1.5	115[abc]	94.4[a]	55.5[bcd]	52.3[ab]	2.18[ab]	+8[abc]
8.0	6.3/7.2	1.5	93[d]	86.1[abc]	55.4[bcd]	47.8[b]	1.95[b]	–154[bcd]
11.5	6.3/7.2	1.5	74[e]	73.8[bc]	53.0[d]	40.4[c]	1.95[b]	–228[d]
5.7	10.1/11.5	1.5	119[ab]	90.1[ab]	58.5[ab]	52.8[ab]	2.25[ab]	+33[ab]
8.0	10.1/11.5	1.5	118[ab]	94.0[a]	56.8[abc]	53.5[ab]	2.19[ab]	–28[abcd]
11.5	10.1/11.5	1.5	101[bcd]	84.9[abc]	58.0[ab]	49.6[ab]	2.12[ab]	–97[abcd]
5.7	6.3/7.2	2.4	130[a]	97.6[a]	57.7[ab]	56.3[a]	2.28[ab]	+71[a]
8.0	6.3/7.2	2.4	97[cd]	80.2[abc]	55.8[abcd]	48.0[b]	2.05[ab]	–90[abcd]
11.5	6.3/7.2	2.4	77[e]	71.0[c]	54.3[cd]	39.7[c]	2.11[ab]	–199[cd]
5.7	10.1/11.5	2.4	130[a]	97.[a]	58.9[a]	57.3[a]	2.24[ab]	+73[ab]
8.0	10.1/11.5	2.4	120[ab]	90.5[ab]	57.3[abc]	52.0[ab]	2.36[a]	+23[ab]
11.5	10.1/11.5	2.4	107[bcd]	90.9[ab]	58.0[ab]	52.6[ab]	1.99[b]	–89[abcd]
Pooled SEM			5	4.5	0.8	1.8	0.08	51
Main effects								
Isoleucine (g/kg)								
5.7			123[a]	94.8[a]	57.6[a]	54.7[a]	2.24[a]	46[a]
8.0			107[b]	87.7[b]	56.3[b]	50.4[b]	2.14[ab]	–62[b]
11.5			90[c]	80.2[c]	55.9[b]	45.7[c]	2.04[b]	–153[c]
Pooled SEM			3	2.4	0.4	1.0	0.04	26
Valine/leucine (g/kg)								
6.3/7.2			98[b]	83.9[b]	55.3[b]	47.5[b]	2.09[b]	–99[b]
10.1/11.5			116[a]	91.3[a]	57.9[a]	53.0[a]	2.19[a]	–14[a]
Pooled SEM			3	2.0	0.3	0.8	0.03	23
Tryptophan (g/kg)								
1.5			103[b]	87.2	56.2	49.5	2.11	–77
2.4			110[a]	87.9	57.0	51.1	2.18	–35
Pooled SEM			3	2.0	0.3	0.8	0.03	23
Interactions (P <)								
Isoleucine × valine/leucine			0.001	0.05	0.05	0.001	0.05	NS

[a–e]Means with the same superscript within a column do not differ significantly ($P < 0.05$).

[1]Results are means with n = 12 per treatment.

factors. When F-values were statistically significant ($P < 0.05$), means were compared by the Newman-Keuls test. To test the correlations among feed consumption, daily egg mass, and BW change, linear correlation analysis was performed.

RESULTS

All performance parameters of the laying hens were affected by the dietary isoleucine concentration and by the concentrations of valine + leucine (Table 2). Significant interactions were observed between the concentration of isoleucine and the concentrations of valine + leucine for all tested parameters except body weight change. At the low concentration of valine + leucine, an increase in the dietary isoleucine concentration led to a significant reduction in performance parameters. Increasing the isoleucine concentration from 5.7 to 11.5 g/kg diet reduced feed consumption by 38%, egg production by 25%, egg weight by 5%, and daily egg mass by 26%. The feed conversion ratio for egg mass on the other hand improved, probably as a result of the marked reduction in BW. At the high concentration of valine + leucine, an increase in the dietary isoleucine concentration had less effect on all performance parameters than at the low concentration of valine + leucine. Increasing the isoleucine concentration from 5.7 to 11.5 g/kg diet reduced feed consumption by 16%, egg production by 6%, egg weight by 1%, and daily egg mass by 7%.

The dietary tryptophan concentration also had a significant effect on feed consumption. At the high concentration of dietary tryptophan, feed consumption was 6% higher than at the low concentration of dietary tryptophan. The parameters egg production, egg weight, daily egg mass, feed/egg mass, and BW change were not different between the dietary tryptophan concentrations. Interactions between the isoleucine concentration and the tryptophan concentration did not occur. There were significant correlations between feed consumption and BW change (Figure 1) and between feed consumption and daily egg mass (Figure 2).

Table 3 shows the concentrations of different free amino acids in the plasma in relation to the dietary concentrations of isoleucine, leucine, valine, and tryptophan. As expected, the strongest treatment response was recorded for the concentrations of the amino acids isoleucine, valine, and leucine. In contrast, the concentration of tryptophan in plasma was not influenced by any of the dietary treatment factors. The effect of the dietary isoleucine concentration on the concentration of isoleucine in plasma was dependent on the dietary level of valine + leucine but not on the dietary tryptophan concentration. At low

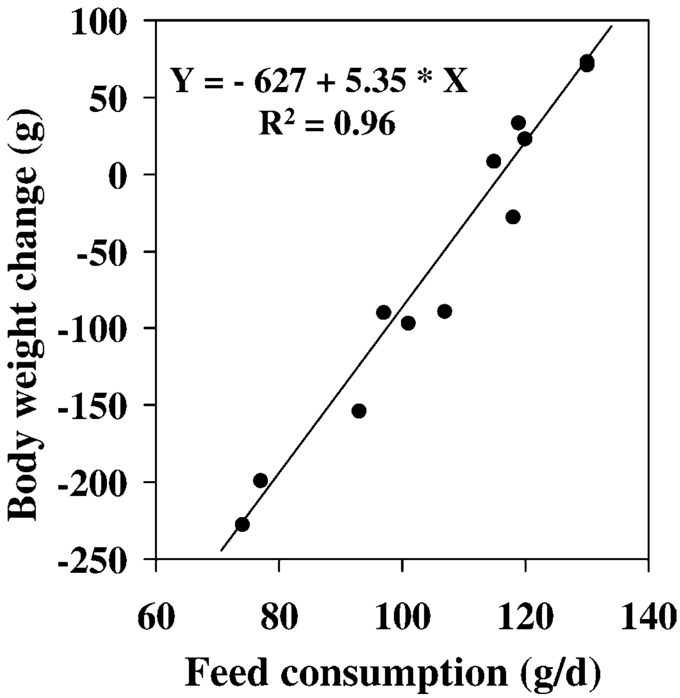

FIGURE 1. Relationship between feed consumption and body weight change of laying hens (y = body weight change, g; x = feed consumption, g/d).

levels of valine + leucine, increase of the dietary isoleucine concentration led to a marked dose-dependent rise in the isoleucine concentration of the plasma. At high dietary levels of valine + leucine, increasing the dietary isoleucine supply did not alter the plasma isoleucine concentration. The dietary isoleucine concentration had no effect on the concentrations of valine, leucine, phenylalanine, methionine, or tryptophan in plasma. In contrast, the concentration of lysine in plasma was significantly reduced by high dietary isoleucine concentration.

Increasing the dietary concentrations of valine + leucine in the diet significantly increased the concentrations of those amino acids and of phenylalanine and lysine. In contrast, increasing the dietary tryptophan concentration did not alter the concentration of any of the amino acids measured in plasma.

DISCUSSION

This study shows that an excessive concentration of isoleucine in laying hen diets leads to a marked performance depression, which can be significantly alleviated by increasing the concentrations of the two other branched-chain amino acids, leucine and valine. This observation confirms the existence of antagonisms among these three amino acids, which has also been described in other studies (Sauberlich, 1961; D'Mello and Lewis, 1970; Tuttle and Balloun, 1976; Shinnick and Harper, 1977). A major factor in this phenomenon is probably the competition between isoleucine and other, structurally similar, amino acids for transfer into the brain across the blood-brain barrier. It has been shown in rats (Peng et al., 1973) and broilers (Harrison and D'Mello, 1986) that an excess of one branched-chain amino acid leads to depletion of other structurally similar amino acids in the brain, causing secondary anorexia. It is likely that the increase in the isoleucine concentration in the blood due to a high dietary isoleucine supply and low levels of valine + leucine inhibits transfer of leucine, valine, and other structurally similar amino acids into the brain, resulting in a significant reduction of the feed consumption as a secondary effect. The finding of high correlations between feed consumption and performance parameters suggests that the lower feed consumption is probably also the main factor responsible for the depressant effect on performance caused by excess dietary isoleucine. A pair-feeding regime, in which animals of all the treatment groups receive identical amounts of food, would be helpful in distinguishing between the effects of an excess intake of isoleucine per se and the effects of reduced feed consumption.

In the present study, basal diets were supplemented with free amino acids to study the interaction between isoleucine and valine + leucine. Free amino acids are more available than protein-bound amino acids (Izquierdo et al., 1988; Han et al., 1990). It is therefore assumed that in practical diets with higher protein concentrations, antagonism between isoleucine and valine + leucine will appear at greater concentrations of those amino acids than those found in the present study.

Studies in broilers have shown that excess branched-chain amino acids are associated with reduced formation of serotonin in the brain (Harrison and D'Mello, 1986).

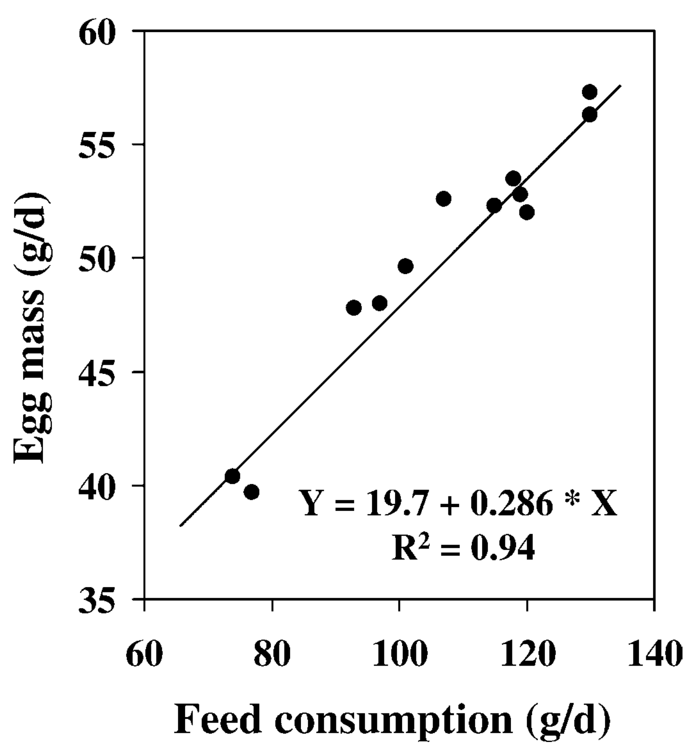

FIGURE 2. Relationship between feed consumption and daily egg mass of laying hens (y = daily egg mass, g/d; x = feed consumption, g/d).

TABLE 3. Concentrations of free amino acids in plasma of laying hens receiving diets with various concentrations of isoleucine, valine + leucine, and tryptophan from 25 to 28 wk of age[1]

Dietary treatment									
Isoleucine (g/kg)	Valine/leucine (g/kg)	Tryptophan (g/kg)	Isoleucine (μmol/L)	Valine (μmol/L)	Leucine (μmol/L)	Phenylalanine (μmol/L)	Lysine (μmol/L)	Methionine (μmol/L)	Tryptophan (μmol/L)
5.7	6.3/7.2	1.5	84^{bc}	162^{bc}	144^{ab}	75	310	81^{ab}	28
8.0	6.3/7.2	1.5	100^{b}	144^{c}	133^{b}	72	306	78^{ab}	24
11.5	6.3/7.2	1.5	141^{a}	157^{bc}	136^{b}	76	317	84^{a}	25
5.7	10.1/11.5	1.5	73^{c}	213^{ab}	158^{ab}	87	384	80^{ab}	28
8.0	10.1/11.5	1.5	80^{bc}	211^{ab}	155^{ab}	86	355	78^{ab}	26
11.5	10.1/11.5	1.5	79^{bc}	171^{bc}	139^{b}	79	289	63^{b}	22
5.7	6.3/7.2	2.4	73^{c}	149^{c}	132^{b}	77	327	74^{ab}	25
8.0	6.3/7.2	2.4	100^{bc}	139^{c}	131^{b}	84	328	76^{ab}	25
11.5	6.3/7.2	2.4	124^{a}	155^{bc}	133^{b}	75	299	83^{a}	27
5.7	10.1/11.5	2.4	72^{c}	188^{abc}	146^{ab}	80	368	77^{ab}	26
8.0	10.1/11.5	2.4	92^{bc}	231^{a}	174^{a}	88	423	82^{ab}	26
11.5	10.1/11.5	2.4	81^{bc}	174^{bc}	134^{b}	76	314	68^{ab}	25
Pooled SEM			30	54	29	15	103	14	1
Main effects									
Isoleucine (g/kg)									
5.7			75^{c}	178	145	80	347^{a}	78	27
8.0			93^{b}	181	149	82	353^{ab}	79	25
11.5			107^{a}	164	136	77	305^{b}	74	25
Pooled SEM			4	8	4	2	15	2	1
Valine/leucine (g/kg)									
6.3/7.2			104^{a}	151^{b}	135^{b}	77^{b}	315^{b}	79	26
10.1/11.5			80^{b}	198^{a}	151^{a}	83^{a}	356^{a}	75	25
Pooled SEM			3	6	3	2	12	21	
Tryptophan (g/kg)									
1.5			93	177	144	79	327	77	25
2.4			91	172	142	80	343	76	26
Pooled SEM			4	6	4	2	12	2	1
Interactions (*P* <)									
Isoleucine × valine/leucine			0.001	0.01	0.05	NS	NS	0.001	NS

[a–c]Means with the same superscript within a column do not differ significantly ($P < 0.05$).

[1]Results are means with n = 12 per treatment.

This finding led us to suspect that a diminished absorption of tryptophan into the brain in the presence of excess isoleucine resulting in a reduced formation of serotonin probably plays a major role in lowering of feed consumption. This supposition was not confirmed, however. The observation that increasing the dietary tryptophan concentration did not alleviate the effects of excess isoleucine suggests that tryptophan and its metabolites do not play a primary role in the reduced feed consumption at high concentrations of dietary isoleucine.

At the high dietary concentration of valine + leucine, an excessive supply of isoleucine did not lead to a significant increase in the blood plasma isoleucine level, probably due to increased oxidation of the three branched-chain amino acids as a result of the increased leucine intake. High intake of leucine stimulates the activity of branched-chain α-keto acid (BCKA) dehydrogenase, the key enzyme involved in degradation of all three branched-chain amino acids (Harris et al., 2001). The performance-lowering effect of excessive leucine is therefore partially due to increased oxidation of valine and isoleucine and the resulting deficiency of these amino acids (Smith and Austic, 1978; Calvert et al., 1982; Block and Harper, 1984). Excess isoleucine does not apparently stimulate BCKA dehydrogenase activity because at normal intake levels of leucine and valine the concentrations of these amino acids in plasma were independent of the dietary isoleucine concentration. In rats, too, a high isoleucine intake does not lead to enhanced oxidation of valine and leucine (Shinnick and Harper, 1977; Block and Harper, 1984). An excess of isoleucine does not presumably cause a secondary deficiency of valine + leucine, but the reduced concentrations of valine + leucine in plasma clearly indicate that at high dietary levels of valine + leucine a high dietary concentration of isoleucine seems additionally to stimulate the activity of BCKA dehydrogenase.

Our study, moreover, shows that increasing the dietary tryptophan concentration from 1.5 to 2.4 g/kg diet did not increase the concentration of this amino acid in plasma or increase the daily egg mass of laying hens. This finding demonstrates that dietary tryptophan at 1.5 g/kg diet, corresponding to a tryptophan intake of 175 mg, is sufficient for maximum daily egg mass. This result agrees with other published studies. Harms and Russell (2000) calculated a daily tryptophan requirement of 149 mg for a daily egg mass of 50 g. The recommendations based on the study by Jensen et al. (1990) are approximately 124 to 168 mg tryptophan daily. The National Research Council (1994) recommends a daily tryptophan intake of 175 mg for brown-egg layers. Interestingly, an increase of dietary tryptophan concentration from 1.5 to 2.4 g/kg significantly increased the feed consumption of laying hens.

This effect could be due to the function of tryptophan as a precursor of serotonin. It is well known that serotonin, which is formed in the brain, influences feed consumption of animals (Tackman et al., 1990; Mullen and Martin, 1992).

Our study, in conclusion, demonstrated the existence of pronounced antagonisms between the amino acids isoleucine and valine + leucine. Interactions between tryptophan and isoleucine, however, could not be detected.

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